
# Agricultural Biotechnology Support Project

## ANNUAL TECHNICAL REPORT

**JANUARY 1 – DECEMBER 31, 1999**

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# CONTENTS

# EXECUTIVE SUMMARY

## Introduction

This report outlines the activities and achievements of the Agricultural Biotechnology Support Project (ABSP) [DAN-A-00-00126-00 and 263-0240-G-00-6014-00] for the period from January through December 1999.

The primary goal of ABSP is:

***To improve the capacity and policy environment for the use, management, and commercialization of agricultural biotechnology in developing countries and transition economies.***

This goal is to be achieved by meeting the following two objectives:

*1) Establishment of a policy framework in developing countries and transition economies which promotes the use, management and commercialization of biotechnology by both host country and multinational agribusiness and research institutions.*

2) *Improvement of marketed crops through strategic research partnerships between the US and developing country public and private sectors.*

# Achievements Against Objectives January 1 – December 31, 1999

## General Management Issues

### *Appointment of External Board of Directors*

Selection of the ABSP External Board of Directors by the USAID project officer with assistance from ABSP was completed by February. The first board meeting was held in Washington DC in June 1999.

The members of the Board of Directors are as follows:

- **Maria Jose Sampaio,** Cornell University
- **Gary Toenniessen,** Deputy Director, Agricultural Sciences, The Rockefeller Foundation
- **Sally Van Wert,** Manager, Regulatory Affairs, AgrEvo
- **Mark Condon,** Vice President, International Marketing, American Seed Trade Association
- **Donald Plucknett,** Agricultural Research and Development International
- **Michael Schechtman,** Biotechnology and Scientific Services, USDA

### *Relocation of ABSP Management Team*

The ABSP Management Team were finally relocated to the Institute of International Agriculture (IIA) at MSU in November 1999. The management team had desired this move for a number of years in order to improve communications. Unfortunately a serious fire in the Institute on December 31, 1999 completely destroyed the ABSP management offices, necessitating another move to alternative accommodation on campus. Further details on the fire and its ramifications for ABSP will be given in the Impact Report (due July 2000).

### *Execution of stand-alone grant with USAID/Cairo and AGERI, Egypt*

Coordination with USAID/Cairo and its partner institution in Egypt, the Agricultural Genetic Engineering Research Institute (AGERI) resulted in the successful execution of its stand-alone grant with the mission in October 1999. This grant was almost an entire year overdue, and our sub grantees had been eagerly awaiting funds to begin their research.

### *Development of ABSP contact database*

The Management Team have upgraded and improved the ABSP database management system, including an improved contact database of expertise in all fields of agricultural biotechnology. Related to this, the Management Team also began the process of identifying expertise in agricultural biotechnology, both internal to MSU and throughout the US, as a component of laying the groundwork for future USAID-funded programs in agricultural biotechnology. A full campus survey of expertise will be conducted in early 2000.

### *Submission of ABSP annual technical report to USAID*

The annual technical report was submitted to USAID during Quarter 2 1999.

### *Submission of ABSP annual impact report to USAID*

Annual impact report was submitted to USAID during Quarter 3 1999.

# Establishment of Policy Framework Activities

## Management Issues

### *Execution of subagreements with cooperating institutions*

This includes two institutions that are focused on policy efforts, CABI and Virginia Tech. Each institution provided ABSP with an annual work plan and execution of sub agreements occurred on schedule early in 1999. All subgrants to ABSP partners (Cornell University, UT Dallas and Ohio State University) were initiated under the USAID/Cairo grant in October/November 1999.

## Intellectual Property Rights

### *East Africa IPR Workshop*

ABSP held a workshop on *The Impact of Intellectual Property Rights on International Trade and Agriculture in East Africa* in Kampala, Uganda from January 18-20, 1999. The Ugandan Council for Science and Technology (UNCST) assisted ABSP in the local organization of the workshop. Additional funds for the support of regional participants to attend the meeting were obtained from the Technical Center for Agricultural and Rural Cooperation (CTA, Netherlands), the Rockefeller foundation and Monsanto. Over 70 participants attended the workshop from Ethiopia, Kenya, Nigeria, Tanzania, Uganda, Zambia, Zimbabwe, Switzerland, United Kingdom, France, United States, Costa Rica, South Africa, and the Netherlands. A full report on this workshop is given in the Technical Report Section.

Dr Barry Greengrass, Vice Secretary-General of UPOV reports that during the workshop he made contact with the responsible persons in Uganda and Tanzania who are now preparing PVP laws. Individuals from these countries have since participated in a two-week course on PVP in Cambridge, United Kingdom, and UPOV are expected to organize national seminars in their countries in January 2000. Valuable contacts were also established Between UPOV and the Commission for Science and Technology of the Organization for African Unity (OAU) in Lagos.

Our collaborators in the Uganda Council for Science and Technology have reported the following impacts that they attribute to be a direct result of the IPR Workshop:

- UNCST interaction with other complementary national institutions handling IPR issues, has tremendously increased.
- UNCST has also noted an increased interest by the public in IPR matters, particularly the local media.
- UNCST together with the National Agricultural research Organisation, Ministry of Justice, Forestry Department, NEMA, Makerere University, Uganda Seed Project, Ministry of Agriculture Animal Industry and Fisheries, has embarked on drawing up Plant Variety Protection Legislation for Uganda.

### *Collaborations with ISNAR*

ABSP continued collaborations with the International Service for National Agricultural Research (ISNAR) in its programs to assist developing countries in the general management of biotechnology within an overall agricultural research portfolio.

ABSP Director, Dr. Catherine Ives was a contributing author to the book *Managing Agricultural Biotechnology*: *Addressing Research Program Needs and Policy Implications Biotechnology in Agriculture Series, No. 23*. Edited by Joel I Cohen, International Service for National Agricultural Research (ISNAR 1999.). The chapter was co-authored by Catherine L. Ives, Karim M. Maredia and Frederic Erbisch and was titled: *International Collaboration: Intellectual Property Management and Partner-Country Perspectives*.

Dr Ives gave a lecture at a course sponsored by ISNAR in the Philippines in November 1999. Participants to the course originated from several Asian countries including Indonesia, Thailand, Vietnam, and the Philippines. ABSP used this opportunity to discuss the importance of incorporating Intellectual Property Rights (IPR) within international research collaborations involving biotechnology. This visit led to greater linkages with CGIAR Centers, specifically IRRI through invited lectures on IPR management. As a result of this visit Dr. Fred Erbisch was also invited back to the region to speak at a conference in February 2000.

The visit also provided opportunity for increased collaboration with the support of USDA. This will be highlighted in a subsequent report. Additionally, ABSP explored the possibility of working with the Philippine government in the development of a risk assessment workshop for members of the Philippine Biosafety Committee and promoting continued collaboration with ISNAR in MSU-sponsored short courses on IPR management and food safety.

ABSP was also able to provide assistance to the Philippines in drafting their commercial guidelines by recommending the services of Mrs Muffy Koch (Innovation Biotechnologies) and providing relevant contract information.

### *Assistance to DPVCTRF, Morocco with implementation of PVP law*

Dr Ives visited Morocco in May 1999, to lecture at a conference on biotechnology and to determine the current operational capacity of the Plant Variety Protection office at the DPVCTRF. (In 1998, ABSP assisted the DPVCTRF in implementing their new PVP law by the purchase and set-up of a computer system). This trip was an opportunity to determine the demand for this office and its current capabilities as well as any additional assistance that may be required, and resulted in an opportunity to access PL480 funds from USDA for biosafety capacity building. A proposal for funding was subsequently drafted and sent to the DPVCTRF for comment and submission in July 1999. A further version was sent for comments in December 1999. DPVCTRF has to our knowledge not yet responded to this proposal.

### *Assistance to Indonesia in IPR and Technology Transfer*

During 1999 ABSP worked closely with collaborators in Indonesia who requested our assistance in a number of policy areas. In June 1999 the Indonesian Minister of Agriculture inaugurated the Intellectual Property and Technology Transfer Office (IPTTO), which will be under the Agricultural Research Foundation, belonging to the Agency for Agricultural Research and Development (AARD). The office will serve as Indonesia's legal and regulatory technology transfer arm in agriculture. Drs. Maredia and Erbisch, ABSP's Technology Transfer Coordinator and Director of MSU's Office of Intellectual Property, spoke at a conference in Indonesia in September, 1999 on

the transfer of agricultural biotechnology and commercialization and were able to provide technical assistance to the IPTTO to assist them in developing general policies and procedures for the office. Within 3 months of its operation, KIAT has executed 5 license agreements to commercialize a wide range of technologies developed by the AARD institutions.

According to Dr. Acmad Fagi, Secretary General of the AARD, this office is the direct result of training received in IPR and Technology Transfer at MSU via the short course. Ketty Karyati, who has received training as part of ABSP's capacity building efforts with Indonesia, will be the administrator of the office as the secretary. KIAT has expressed interest in running an in-country IPR workshop to educate key scientists and various AARD institutions. In addition, MSU's draft IP policy was shared with KIAT to be used as a basis for developing a system-wide policy in IP.

Dr. Didiek Hadjar from the Estate Crops Research Institute attended the MSU IPR and Technology Transfer Course in 1998. He has since co-founded a new organization called the Indonesian Inventor Society and is serving as President. Again, this organization was developed as a direct result of Dr. Didiek's participation in the course. There have been several biofertilizer/biofungicide technologies patented with the assistance of this organization and are in various stages of commercialization. A copy of the brochures on these technologies is available (in Indonesian).

Indonesia has developed a draft of a Plant Variety Protection, which was released at the end of 1999.

### *Drafting of Plant Variety Protection Legislation in the Philippines*

Ms. Conception Magboo from the Philippines attended the IPR Internship Program at MSU during the summer of 1999. Ms. Magboo is now a member of the team that is drafting the Plant Variety Protection Act in the Philippines. Her participation in the IPR internship program was sponsored by ISNAR.

## Biosafety

### *Collaborations with SEI BIO-EARN*

ABSP has continued coordination of future biosafety policy efforts with the Stockholm Environmental Institute (SEI). Dr. Ives was appointed a Steering Committee member to the East Africa Regional Network on Biosafety and Biotechnology which is being funded by SADEC through SEI.

Dr. Ives has worked with the Stockholm Environment Institute as a member of the Steering Committee (SC) for the East African Regional Network on Biotechnology, Biosafety and Biopolicy (EARN). Dr Ives attended the SC in Tanzania in October, finalizing research projects and exploring policy-building efforts. EARN and ABSP have continued to collaborate on biosafety capacity building, mainly through the sharing of risk assessment case studies. Dr. Ives has served as a linkage between EARN and any proposed ASARECA Biotechnology Program to ensure efficiencies between the donor organizations

### *ICGEB Biosafety Workshop*

Dr Andrea Johanson attended the International Centre for Genetic Engineering and Biotechnology (CGEB) Workshop: *Science & Policy In Risk Assessment Of Transgenic Plants: A Case Study Approach* held at the ICGEB in Trieste in April 1999. The purpose of attending the course was to increase ABSP capacity in biosafety issues, and to assess

the usefulness of the course for ABSP collaborators to attend in future years. It was also valuable in making contact with other international organizations involved in building capacity in biosafety in developing countries.

### *Assistance to Indonesia in Biosafety*

ABSP has assisted collaborators in Indonesia who have expressed interest in accessing ABSP resources for help in evaluating applications for field-testing of transgenic crops. Indonesia's newly established National Biosafety Committee now has to implement its national regulations.

Indonesia's Biosafety Committee has now given deregulated status to 5 transgenic crops (Bt cotton, Bt corn, Roundup Ready cotton, Roundup Ready corn, and Roundup Ready soybean from Monsanto) for unconfined multi-location trials. They are currently conducting greenhouse trials of Bt corn from Pioneer Hi-Bred, and anticipate field trials in 2000. Other crops are currently being tested (see table for summary). ABSP has also worked closely with both the Indonesian Biosafety Committee and USAID's Biosafety Committee to obtain approval for the field-testing of transgenic potatoes developed in collaboration with MSU and the Research Institute for Vegetables (RIV) in Indonesia. The potatoes are currently awaiting approval by the Indonesian Biosafety (see Research Collaborations below).

ABSP sponsored two participants, Drs. M. Herman and A. Hidayat, to attend MSU's Food Safety Course in July 1999. They are currently members of the national committee to draft national food safety guidelines and legislation for Indonesia.

**Current status of biosafety testing for transgenic crops in Indonesia**

| Crops | Traits | Institution/ Private Co. | Biosafety containment test | Confined field test | Multilocation tests |
|---|---|---|---|---|---|
| Bt corn | Resistant to ACB | Pioneer | Being conducted | – | – |
| Bt corn | Resistant to ACB | Monsanto | Were conducted | Were conducted | – |
| Pin II Corn | Resistant to ACB | RIFCB/ABSP | Being conducted | – | – |
| RR Corn | Resistant to *glyphosat* | Monsanto | Were conducted | Were conducted | Were conducted |
| Bt cotton | Resistant to CBW | Monsanto | Were conducted | Were conducted | – |
| RR cotton | Resistant to *glyphosate* | Monsanto | Were conducted | Were conducted | – |
| Peanut | Resistant to Pstv | RIFCB/ACIAR | Will be conducted | – | – |

| | | | | | |
|---|---|---|---|---|---|
| RR soybean | Resistant to *glyphosate* | Monsanto | Were conducted | Were conducted | Will be conducted |
| Bt potato | Resistant to PTM | RIVC/RIFCB/ CRIFC/MSU | Were conducted | Will be conducted | – |
| Bt and GNA rice | Resistant to RSB and BPH | CRIB, IIS | Being conducted | – | – |
| Bt rice | Resistant to RSB | RIFCB | Being conducted | – | – |

ABSP = Agricultural Biotechnology Support Project; ACB = Asian corn borer; ACIAR = Australian Center for International Agricultural Research; BPH = Brown Plant Hopper; Bt = *Bacillus thuringiensis*; CBW = Cotton Bollworm; CRIB = Central Research Institute for Biotechnology; CRIFC = Central Research Institute for Food Crops; GNA = *Galanthus nivalis* agglutinin (snowdrop lectin); IIS = the Indonesian Institute for Sciences; MSU = Michigan State University; Pstv = peanut stripe virus; PTM = potato tuber moth; RIFCB = Research Institute for Food Crops Biotechnology; RIVC = Research Institute for Vegetable Crops; RR = Roundup Ready; RSB = Rice Stem borer.

### *Linkage building and biosafety in Southern and Eastern Africa*

In February 1999 ABSP sponsored and assisted the participation of Dr. Joel Cohen (ISNAR) and Mrs Muffy Koch (Innovation Biotechnology) in an assessment of biosafety needs and capabilities in East and Southern Africa and on the feasibility of a biotechnology program and regional biosafety regime under the guidance of ASARECA. This activity was sponsored by USAID/Africa Bureau and was undertaken in collaboration with the United National Development Program (UNDP) and ISNAR. The Association for the Strengthening of Agricultural Research in East and Central Africa (ASARECA) requested this assessment as an initial step in designing a regional approach to biosafety regulation.

Following this assessment, Dr. Andrea Johanson traveled to Nairobi in September 1999, to attend the Committee of Directors Meeting of the Association for Strengthening Agricultural Research in East and Central Africa (ASARECA). Discussions were held with ISNAR and UNDP on possible capacity building efforts in biosafety and biotechnology in the region. Since then, ABSP has continued to work with ASARECA to provide information on the current status of biotechnology in developed countries, technologies that could be easily adapted, etc. in order to assist these countries in developing and using biotechnology. This effort is supported by the Africa Initiatives funds from USAID/Africa Bureau.

### *Biosafety Workbook*

ABSP have begun planning on the development of a biosafety workbook. A meeting was held at MSU in January, 1999, with the ABSP Director, Assistant Director, Dr. Pat Traynor, and Dr. Josette Lewis, USAID Project Officer to discuss the purpose of the workbook and to develop a preliminary table of contents. The concept note for the workbook is appended to the Technical Report.

### *Assistance in Egypt IPR/Technology Transfer Workshop*

ABSP and Michigan State University in collaboration with the Ministry of Agriculture and Land Reclamation, the Ministry of Trade and Supply, the Agricultural Policy Reform Program/DAI, the SIPRE project (Strengthening Intellectual Property Rights in Egypt) and the USAID/Cairo organized a one-day seminar and a four-day course on Intellectual Property Rights (IPR). The seminar and the course were held at the International Egyptian Agriculture Center in Cairo from April 18 - 22, 1999. Dr. Frederic Erbisch, Dr. Catherine Ives, Dr. Karim Maredia from Michigan State University, Prof. John Barton from Stanford Law School, and Dr. Marsha Stanton from the US Department of Agriculture served as the resource persons for the seminar and the course.

The purpose of the seminar was to create greater awareness and to educate senior policy makers and administrators from public and private sectors in Egypt on various issues for IPR. The four-day course targeted the policy personnel, administrators and scientists who will actually be involved in the implementation of the IPR laws and policies in Egypt. The course provided hands-on experience on various aspects of IPR and technology transfer as they relate to agriculture including plant variety protection, patents, plant breeders' rights, copy rights, trade marks, trade secrets, licensing and license agreements, impact of IPR on trade and capacity building in IPR. Over 100 participants attended the seminar and over 75 participants attended the course.

### *Haas Business Assessment for AGERI, Egypt*

In May 1999, an assessment team from the University of California-Berkeley's (UCB) Haas Business School conducted an assessment on the Commercialization Prospects for the Agricultural Genetic Engineering Research Institute (AGERI) in Egypt. The report indicated that AGERI could not be self-supporting if USAID/Cairo funds cease as scheduled in 2001. It recommended that privatization plans be slowed down, and that AGERI develop appropriate strategic marketing, resource allocation and business strategies to promote an effective transition to a self-sustaining institution.

Dr. Catherine Ives traveled to the University of California at Berkeley to hear the presentation of the final report of the UCB assessment team on their assessment of the potential privatization of AGERI. Additional work began on the follow-up to this report and selecting the next assessment team from UC-Berkeley for the formulation of a business plan for AGERI. AGERI and USAID/Cairo are expected to use the results of this current report in formulating future support and institute management changes.

Due to the findings of the report, USAID/Cairo has begun the process of developing a strategy to endow a research foundation to support competitive research proposals from the agricultural community in Egypt

### *Assistance to AGERI in establishing Technology Transfer Office*

Dr. Magdy Madkour and Dr. Mohamed Eid from Egypt visited MSU in May 1999 to discuss establishment of the Office of Technology Transfer and Intellectual Property at AGERI. They interacted closely with the MSU Office of Intellectual Property Rights (OIP). MSU provided them with copies of the OIP's bulletins, educational materials and university policy information. Based on this information Dr. Eid drafted an Intellectual Property Rights (IPR) policy for the Agricultural Genetic Engineering Research Institute (AGERI). A copy of this policy was sent to MSU for review and comments, and forwarded to USAID. AGERI now has an OTTIP policy as well as draft MTA's and Licensing Agreements. This IP policy will serve as the basis for the technology transfer program at AGERI and eventually all the agricultural research centers in the Ministry of Agriculture.

### *Assistance to USAID Africa Bureau in Grades and Standards*

Through funding from USAID's Africa Bureau ABSP has begun to explore issues of Grades and Standards, Food Safety and Biosafety in African Agriculture. Biosafety Initiatives are linked to our ASARECA activities (see previous item). For grades and standards, ABSP in collaboration with MSU's Institute for Food and Agricultural Standards, developed a scope of work to assess the need for grades and standards in 2 African countries. We are currently working with the Africa Bureau to identify countries, commodities and constraints.

# ABSP Research Collaborations

*Please Note: Only research highlights are listed here. Full Technical Reports for all research collaborations are appended to this Executive Summary.*

## *Cucurbits*

**Cornell University**

Breeding of squash, melon and cucumber at Cornell University has been expanded to include resistance to four major viruses, and two fungal diseases. The objective is to bring together as many of these resistances as possible to stabilize yield under less or unprotected conditions in tropical areas. A number of new varieties important in the Middle East and other parts of the world have been obtained, and field trials carried out in Ithaca, NY. Resistant plants and progenies have been selected and controlled pollinations made to advance generations and to cross-resistant plants with these new varieties.

A major impact for 1999 has been the establishment of field trials of materials produced under ABSP Phase I not only in Egypt where they had been targeted, but also in a number of additional locations, and for the first time, we have involved the private sector in these testing efforts. Field trials are now underway or planned for in Jordan (Seminis Vegetable Seeds), Morocco (Novartis), Philippines and Indonesia (sister companies of East West Seed Co.), and are in the process of arranging sites in Turkey (Rijk Zwaan) and Tunisia (Seminis Vegetable Seed). Future trials are planned with collaborators in Jordan, Puerto Rico, Costa Rica, Honduras and Brazil.

In late 1999/early 2000 the first licensing agreements for several items produced from support form ABSP were signed. These include multi-virus resistant cucumber and melons, and squash with resistance to powdery mildew.

**1999 Cucurbit Trials**

| Country | Collaborator | Crop/type | Objective |
|---|---|---|---|
| Egypt | Inst of Hort/AGERI | squash,<br>cucumber<br>tropical pumpkin | multivirus resistance<br>and adaptation |
| Philippines | East/West Seed Co. | melon, cucumber, | multivirus + powdery mildew |
| Indonesia | East/West Seed Co. | melon, cucumber, | multivirus + powdery mildew |
| Jordan | Peto/Seminis | squash, cucumber, melon, | multivirus + powdery mildew |

| | | | |
|---|---|---|---|
| Morocco | Rogers/Novartis | squash, cucumber, melon, | |
| Ithaca, NY | | all | all |
| Morocco | Domaine Agricole | | |
| India | Seminis | cucumber, melon | multivirus + multifungal |

**Michigan State University**
The non-regeneration based electrotransformation system has been tested on cucumber and resulted in successful delivery of DNA (as assessed by PCR) to vegetative tissue of cucumber (ca. 10% of the treated plants). So far this has not resulted in transgenic progeny, but modifications of this method are being investigated.

A graduate student was brought to MSU via additional external funding support, thus strengthening linkages.

**AGERI**
AGERI have been successful in introducing a gene for resistance to Zucchini Yellow Mosaic Virus into the Egyptian cultivar Eskandarani using an *Agrobacterium* transformation system. Several families of $R_3$ transgenic squash plants have shown high levels of resistance to ZYMV isolates in the field, and $R_4$ lines are currently being tested.

AGERI report having successfully established a regeneration and transformation system in the Beit Alpha open field cultivar of Cucumber.

## *Potato*

Field trials of the Bt-transgenic potato lines with resistance to potato tuber moth developed at Michigan State University were harvested in Egypt in June 1999. These trials were planted in February 1999, at the CIP research station and at the Agricultural Genetic Engineering Research Institute (AGERI), with the purpose of obtaining field data toward both agronomic performance and resistance to potato tuber moth (PTM) damage to the foliage and tubers. These trials are now in their third year at AGERI and second year at CIP.

Results from this year's field trials were very promising. On average, the percent tuber moth damage was 27 - 28% for the Spunta and Atlantic potato cultivars, whereas the Bt-transgenic lines averaged between 80 - 100% healthy tubers. Other lines showed even greater protection against tuber moth.

During a visit to Egypt, important strategic linkages were established with the Egyptian private and public sector, seed and commercial growers and information was disseminated regarding the potential commercial use of Bt-transgenic potato lines.

In Indonesia, field-testing is pending approval from the Indonesian and USAID Biosafety Committees. ABSP is continuing to work with Indonesia and USAID to receive approval for field-testing in 2000.

The Agricultural Research Center (ARC) of South Africa has expressed interest in testing material in South Africa and work has begun obtaining approvals for field-testing from the appropriate regulatory authorities.

We have an ongoing relationship with the International Potato Center (CIP) to assess the transgenic potatoes developed by MSU researchers. This material has not yet moved into the field. Approvals from USAID's Biosafety Committee and the Peruvi an government will be required before this occurs.

Commercialization of the various potato varieties and constructs will require negotiations with holders of various intellectual properties (i.e. promoters, drug resistance markers, Bt genes, etc). These negotiations will continue throughout the coming year to obtain clear commercialization rights to the material developed at MSU.

Researchers at AGERI, Egypt have succeeded in developing an effective method for regeneration and transformation of potato in their laboratory. Both the popular varieties Spunta and Desiree were amenable to this technique.

### *Tomato*

Work at AGERI, Egypt has succeeded in transforming tomato with resistance to the economically damaging Tomato Yellow Leaf Curl Virus (TCLV) using a 'sui strategy. Seeds from these plants are currently being collected for further testing.

### *Maize*

Collaboration has continued between Pioneer HiBred and AGERI in the training of Egyptian scientists in the technical skills needed for the rearing of corn borers, transformation of maize varieties, and the study of the insecticidal proteins of *Bacillus thuringiensis.*

Several new putative promoters have been isolated from maize and are undergoing further testing. A patent on some of these new promoters was filed in late 1998.

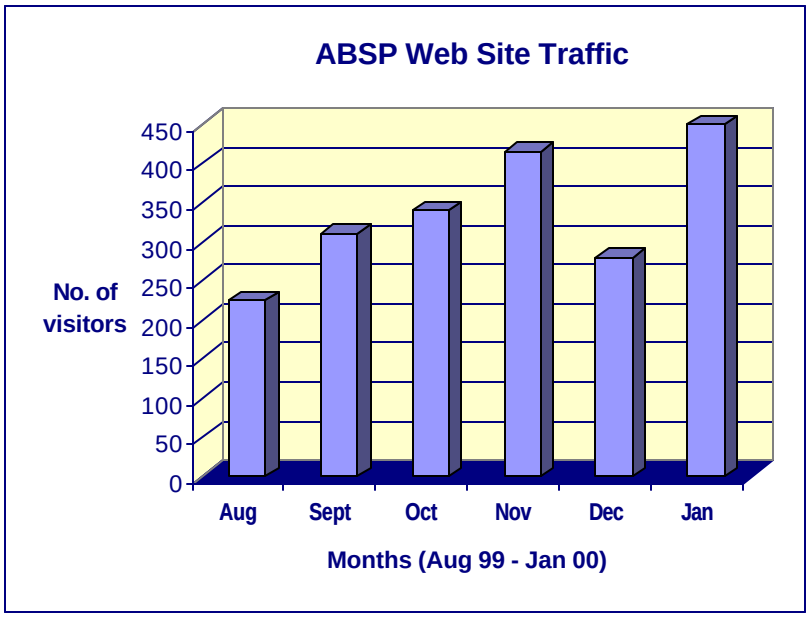
A US patent (5986177A) on *B. thuringiensis Isolate with Broad Spectrum Activity* was issued to AGERI on November 16, 1999.

## ABSP Networking & Communications

### *Development of ABSP's World Wide Web pages*

In June 1999 the Website of the Agricultural Biotechnology Support Project (http://www.iia.msu.edu/absp/) was chosen as HMS Beagle's "Web Pick of the Day". HMS Beagle is a biweekly 'webzine' for biological and medical researchers, with a daily digest of the highest-quality Web resources and published material. Subsequent to receiving this award the ABSP page was featured on the HMS Beagle home page with a link to our site. It was then listed on Beagle's "Favorite Web Sites" page for 30 days, as well as permanently catalogued in *BioMedLink* (http://biomedlink.com), their comprehensive, evaluated database of biological and medical websites.

The Website has been updated and expanded in the last 12 months, and has received considerable attention. On average the site is now receiving over 500 visitors per month (see graph).




This increase in traffic is also shown by an increase in the number of inquiries received by ABSP relating to topics on the Website. These inquiries have included:

- A request from an agricultural company in Kuwait for information on where they could obtain tissue culture plantlets of potato, banana and pineapple in order to set up a tissue culture laboratory. ABSP was able to put the company in touch with our previous collaborator Dr Oscar Arias of Agrobiotecnologia de Costa Rica and a successful linkage was made between the two companies.
- Dr Nombasa Tsengwa from South Africa 'discovered' ABSP from the Website and subsequently came to visit us at MSU. Dr Tsengwa has recently been appointed as Biotechnology Manager at the Center for Scientific and Industrial Research in South Africa and was very interested in ABSP's approach to biotechnology management and intellectual property rights issues. We have since been able to link Dr Tsengwa to a US consulting company looking to invest in biotechnology in South Africa.

### *Development of AgbiotechNet*

ABSP has continued to support CABI's *AgbioTechNet* to provide information on technical, regulatory and legal aspects of agricultural biotechnology to our LDC collaborators. ABSP has continued to support our collaborators' subscriptions to AgBiotechNet and increase the number of LDC institutions ABSP supports. See full CABI technical report for more details.

The average number of user sessions per day since the launch in January 1999 increased from around 80 in January 1999 to over 170 in December. *AgBiotechNet*

also has a listserv facility which alerts users to new developments e.g. publication of new review articles. The number of registered listserv members has grown to over 150 since the launch of the service. Over 95% of CABI's printed subscriber base has registered for access to the web site. Approximately 30% of *AgBiotechNet*'s subscribers are located in developing countries.

In 1999 CABI *Publishing* developed relationships with a number of organizations generating content in the field of agricultural biotechnology for developing countries. The first such relationship featured the inclusion of ISAAA *Brief* documents on *AgBiotechNet*. In November 1999 a series of articles commissioned by IFPRI and edited by Gabrielle Persley were added to the site. Three reports from the National Agricultural Biotechnology Council were also added. The reports focus on the following topics:

- *Agricultural Biotechnology: Novel Products and New Partnerships*
- *Resource Management in Challenged Environments*
- *Agricultural Biotechnology and Environmental Quality: Gene Escape and Pest Resistance*

Highlights from Review articles published in 1999 include:

- *Could agricultural biotechnology contribute to poverty alleviation?* (Charles Spillane) ABN 042
- *AGBIOTECH 99: Biotechnology and World Agriculture* (David Massey and David Hemming) ABN 041
- *Public acceptance of genetically engineered food in developing countries: the case of transgenic rice in the Philippines* (Philipp Aerni) ABN 031
- *Agricultural Molecular Biotechnology in South Africa – new developments from an old industry* (Ed Rybicki) ABN 023
- *Technology transfer and licensing of agricultural biotechnologies in the international arena* (Karim M. Maredia, Frederic H. Erbisch, Catherine L. Ives, Andrew J. Fischer) ABN 017
- *The Asian biotechnology market: emerging investment trends* (Sachin Chaturvedi) ABN 012
- *Coupling biotechnology to biodiversity in Africa and the Caribbean* (Senyo Opong) ABN 002

**ABSP's AgBiotechNet subscribers**

As a founding member of *AgBiotechNet* with CABI, ABSP has been able to make this service available to its collaborators, free of charge, for the period of the ABSP project (until September 2001). At this point the following institutions have received such subscriptions from ABSP:

- Uganda National Council for Science and Technology (UNCST), Uganda.
- Agricultural Genetic Engineering Research Institute (AGERI), Egypt.
- Ethiopian Agricultural Research Organization (EARO, Ethiopia.
- ARC-Roodeplaat V.O.P.I, Republic of South Africa.
- National Bureau of Plant Genetic Resources, India.

- Kawanda Agricultural Research Institute Library, Uganda.
- Kenyan Agricultural Research Institute (KARI), Kenya.

In January 1999, Dr Ives made a site visit to CABI, UK to discuss progress in the development of the *AgBioTechNet* site.

## *ABSP Support for Food safety and IPR Training*

ABSP has continued to support individuals to attend MSU sponsored courses on Food safety and Intellectual Property Rights (July 1999).

**IPR and Technology Transfer Internship Program (July 18 - 24, 1999):**

- Dr. Sugiono Moeljopawiro (Indonesia),Research Institute for Food Crops Biotechnology (RIFCB)
- Dr. Tilahun Zeweldu (Ethiopia), Ethiopian Agricultural Research Organization (EARO)
- Dr. Cobus Coetzee (South Africa), Agriculture Research Council (ARC)
- Mr Amr Ageez, (Egypt) AGERI. Also attended AUTM workshop.

**Food Safety Short Course (July 11 - 16, 1999)**

- Dr.Muhammad Herman (Indonesia), Research Institute for Food Crops Biotechnology (RIFCB)
- Dr. Achmad Hidayat (Indonesia), Research Institute for Food Crops Biotechnology (RIFCB)
- Dr. Taymour Nasr El-Din (Egypt), Agricultural Genetic Engineering Research Institute (AGERI)
- Mr. Hisham El-Sheshtawy (Egypt), Agricultural Genetic Engineering Research Institute (AGERI)

## *ABSP participation in conferences/workshops*

ABSP has participated in several high profile conferences, seminars and workshops. These outreach efforts are an important part of increasing ABSP's visibility within the donor community, government and executive branches, and the general public. They have included:

- Dr. Catherine Ives was an invited speaker and chairperson at the Lessons without Borders conference in Ames, Iowa March 18-19, 1999. She presented a case study of public/private partnerships in agricultural research.
- Dr. Catherine Ives was an invited speaker to two meetings of USAID's Board of International Food and Agricultural Development (BOSTID). In February, she discussed "*Public-Private Partnerships: Challenges and Opportunities*." In June, Dr. Ives spoke on "*Capacity Building in IPR for Development of Biotechnology*." These presentations led BOSTID to make a strong statement in support of biotechnology for developing countries, in general, and, in particular, stated that the types of efforts encompassed under ABSP were important keys to the success of adoption of biotechnology by developing countries.

- Dr. Catherine Ives was an invited speaker at the Association of International Agricultural and Rural Development's (AIARD) Capitol Hill Forum in June 1999. *Can Biotechnology Reduce World Hunger*?" which was well received. Participants at the seminar included staff of Congressional Members and Committees. Dr. Ives was interviewed by Voice of America after the presentation
- Dr. Catherine Ives was an invited speaker to a Ford-Rockefeller Foundation Roundtable on March 25, 1999. She gave a brief presentation on the use of biotechnology for developing country agriculture – promises and pitfalls.
- Dr. Catherine Ives was an invited speaker to Rural Week at the World Bank on March 26, 1999 and gave a presentation to World Bank project managers on "*The Growth of Agricultural Biotechnology and its Implications for Developing Country Agriculture – Promises and Pitfalls*." The presentation was well received and has led to other invitations from the World Bank to speak at conferences about agricultural biotechnology and its related policies.
- Dr Andrea Johanson attended the conference "*The Shape of the Coming Agricultural Biotechnology Transformation: Strategic Investment and Policy Approaches from an Economics Perspective*" held at the University of Rome, Tor Vergata June 17-19, 1999. Dr Josette Lewis presented their co-authored paper*: "The Role of Biotechnology Policies and Regulations in Technology Transfer to Developing Countries*." The paper was very well received as one of the few giving actual examples of technology transfer in agricultural biotechnology to developing countries.

# ABSP TRAVEL JANUARY – DECEMBER 1999

| LAST NAME | FIRST NAME | AFFILIATION | COUNTRY | REASON FOR TRAVEL | DESTINATION |
|---|---|---|---|---|---|
| **January** | | | | | |
| Barton | John | Stanford University | USA | E. Africa IPR Workshop | Kampala, Uganda |
| Blakeney | Michael | University of London | UNITED KINGDOM | E. Africa IPR Workshop | Kampala, Uganda |
| Gibbons | Susan | Michigan State University | USA | E. Africa IPR Workshop | Kampala, Uganda |
| Gopo | Joseph | Biotechnology Research Institute (SIRDC) | ZIMBABWE | E. Africa IPR Workshop | Kampala, Uganda |
| Greengrass | Barry | UPOV | SWITZERLAND | E. Africa IPR Workshop | Kampala, Uganda |
| Ives | Catherine | Michigan State University | USA | E. Africa IPR Workshop | Kampala, Uganda |
| Johanson | Andrea | Michigan State University | USA | E. Africa IPR Workshop | Kampala, Uganda |
| Kohi | Yadon | Tanzania Commission for Science & Technology | TANZANIA | E. Africa IPR Workshop | Kampala, Uganda |
| Kunkuta | Musesha | Patents and Companies Registration | ZAMBIA | E. Africa IPR Workshop | Kampala, Uganda |
| Lettington | Robert | | BOTSWANA | E. Africa IPR Workship | Kampala, Uganda |
| Lewis | Josette | USAID | USA | E. Africa IPR Workshop | Kampala, Uganda |
| Manyonga | Richard | Zimbabwe Patents Office | ZIMBABWE | E. Africa IPR Workshop | Kampala, Uganda |
| Mpiri | Daudi | Ministry of Agriculture & Cooperatives | TANZANIA | E. Africa IPR Workshop | Kampala, Uganda |
| Mpofu | Bella | Research and Specialist Services | ZIMBABWE | E. Africa IPR Workshop | Kampala, Uganda |
| Mtetewaunga | Stephen | Ministry of Industries and Trade | TANZANIA | E. Africa IPR Workshop | Kampala, Uganda |
| Mushita | Tonderai | Community Technology Development Trust (CTDT) | ZIMBABWE | E. Africa IPR Workshop | Kampala, Uganda |
| Mwamba | Charles | National Council for Scientific Research | ZAMBIA | E. Africa IPR Workshop | Kampala, Uganda |
| Obongo | Francis | Plant Breeders Association of Kenya | KENYA | E. Africa IPR Workshop | Kampala, Uganda |
| Salazar | Sylvia | SIECA | COSTA RICA | E. Africa IPR Workshop | Kampala, Uganda |
| Sese | Lucas | Kenya Industrial Property Office | KENYA | E. Africa IPR Workshop | Kampala, Uganda |
| Sikinyi | Evans | KEPHIS | KENYA | E. Africa IPR Workshop | Kampala, Uganda |
| Wolson | Rosemary | University of Cape Town | SOUTH AFRICA | E. Africa IPR Workshop | Kampala, Uganda |
| Maredia | Karim | Michigan State University | USA | Biosafety Workshop | Cairo, Egypt |
| Aaouine | Mohamed | Domaine Agricole El Bassatine | MOROCCO | Biosafety Workshop | Cairo, Egypt |
| **February** | | | | | |

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| Koch | Patricia | Innovation Biotechnology | SOUTH AFRICA | Biosafety assessment/Africa | Uganda, Kenya, Tanzania |
| Ives | Catherine | Michigan State University | USA | USAID Meeting | Washington DC |
| **March** | | | | | |
| Maredia | Karim | Michigan State University | USA | Site visit-Lee Bulla | Dallas, TX |
| Ives | Catherine | Michigan State University | USA | Visit Pioneer & Global Agr. Conf. | Ames, Iowa |
| Pett | Walter | Michigan State University | USA | Peru site visit-potato | Cuzco, Peru |
| Johanson | Andrea | Michigan State University | USA | Banana Symposium | Ithaca, New York |
| **April** | | | | | |
| Ives | Catherine | Michigan State University | USA | Am. Cocoa Res. Institute & Penn State | Washington, DC |
| Ives | Catherine | Michigan State University | USA | UC Berkley for AGERI/Egypt project | Berkley, CA |
| Madkour | Magdy | AGERI | EGYPT | Pioneer, IA,; Berkley, CA; Bio '99/WA | & ABSP staff/ MSU |
| Ives | Catherine | Michigan State University | USA | Finalise ABSP/AGERI 3-Yr. Project | Cairo, Egypt |
| Pett | Walter | Michigan State University | USA | Egypt site visits | Cairo, Egypt |
| Johanson | Andrea | Michigan State University | USA | ICGEB Workshop/Transgenic Plants | Trieste, Italy |
| Lacey | Melba | Michigan State University | USA | Grants Meeting | Las Vegas, NV |
| **May** | | | | | |
| Ives | Catherine | Michigan State University | USA | Biodiversity Workshop & other site visits | Ifrane, Morocco |
| MeGeed | Eid | AGERI | EGYPT | Bio '99 Conf-WA/ & MSU visit | Bio "99/WA & MSU |
| Bulla | Lee | University of Texas | USA | Meeting w/Dr. Madkour & ABSP staff | East Lansing, MI |
| **June** | | | | | |
| Coombs | Joseph | Michigan State University | USA | Potato harvest at Egypt site | East Lansing, MI |
| Douches | Dave | Michigan State University | USA | Potato harvest at Egypt site | Giza, Egypt |
| Zarka | Kelly | Michigan State University | USA | In Vitro Biology Conference | New Orleans, LA |
| Barton | John | Stanford University | USA | External BOD Meeting | Washington, DC |
| Condon | Mark | American Seed Trade Association | USA | External BOD Meeting | Washington, DC |
| Erbisch | Fred | Michigan State University | USA | External BOD Meeting | Washington, DC |
| Grafius | Ed | Michigan State University | USA | External BOD Meeting | Washington, DC |
| Grumet | Rebecca | Michigan State University | USA | External BOD Meeting | Washington, DC |
| Ives | Catherine | Michigan State University | USA | External BOD Meeting | Washington, DC |
| Johanson | Andrea | Michigan State University | USA | External BOD Meeting | Washington, DC |

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|---|---|---|---|---|---|
| Lacey | Melba | Michigan State University | USA | External BOD Meeting | Washington, DC |
| Maredia | Karim | Michigan State University | USA | External BOD Meeting | Washington, DC |
| Plucknett | Donald | Agricultural Research & Development International | USA | External BOD Meeting | Washington, DC |
| Schechtman | Michael | USDA | USA | External BOD Meeting | Washington, DC |
| Toenniessen | Gary | The Rockefeller Foundation | USA | External BOD Meeting | Washington, DC |
| Traynor | Patricia | VA Polytechnic Institute & State University | USA | External BOD Meeting | Washington, DC |
| Van Wert | Sally | AgrEvo USA Company | USA | External BOD Meeting | Washington, DC |
| Johanson | Andrea | Michigan State University | USA | ICABR Biotechnology Conference | Rome, Italy |
| Naseem | Anwar | Michigan State University | USA | ABSP Assessment/Philippines | Manila, Philippines |
| Cabrera | Jorge | REMERFI | COSTA RICA | Biotechnology/Biosafety Workshop | Tegucigalpa, Honduras |
| **July** | | | | | |
| El-Sheshtawy | Hisam | AGERI | EGYPT | MSU Food Safety Course | E. Lansing, MI |
| Hidayat | Achmad | Research Institute for Food Crops Biotechnology | INDONESIA | MSU/Food Safety course | East Lansing, MI |
| Ibrahim | Taymour | AGERI | EGYPT | MSU Food Safety Course | E. Lansing, MI |
| Coetzee | Cobus | Agriculture Reseach Council-Fynbos Unit | SOUTH AFRICA | MSU/IPR short course | East Lansing, MI |
| Herman | Muhammad | CRIFC | INDONESIA | MSU/IPR short course | East Lansing, MI |
| Moeljopawiro | Sugiono | RIFC Biotechnology | INDONESIA | MSU/IPR short course | East Lansing, MI |
| Salinas | Cornelio | University of the Philippines | PHILIPPINES | MSU/IPR short course | East Lansing, MI |
| Zeweldu | Tilahun | Agricultural Research Organization (EARO) | ETHIOPIA | MSU/IPR short course | East Lansing, MI |
| **August** | | | | | |
| Erbisch | Fred | Michigan State University | USA | IPR Workshop & site visits | Jakarta & Bogor, Indonesia |
| Maredia | Karim | Michigan State University | USA | IPR Workshop and site visits | Jakarta & Bogor, Inodnesia |
| **September** | | | | | |
| Johanson | Andrea | Michigan State University | USA | ASARECA 1999 Annual Meeting | Nairobi, Kenya |
| Koch | Patricia | Innovation Biotechnology | SOUTH AFRICA | ASARECA Meeting | Nairobi, Kenya |
| Ives | Catherine | Michigan State University | USA | Consultants-Egypt-Haas School of Business | Univ. of Berkeley, CA |
| Sikinyi | Evans | KEPHIS | KENYA | Visit MSU/ABSP researchers and staff | East Lansing, MI |
| **October** | | | | | |
| Johanson | Andrea | Michigan State University | USA | CGIAR Biotechnology Conference | Washington, DC |
| Maredia | Karim | Michigan State University | USA | CGIAR Biotech Conference & Centers Week | Washington, DC |

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| Ives | Catherine | Michigan State University | USA | Egypt meeting w/M. Madkour | Washington, DC |
| Meyer | Terry | Pioneer Hi-Bred International, Inc. | USA | Egypt meeting w/M. Madkour | Washington, DC |
| Ives | Catherine | Michigan State University | USA | BIO-EARN Meeting | Tanzania |
| **November** | | | | | |
| Ives | Catherine | Michigan State University | USA | ISNAR seminar & site visits | Philippines |
| **December** | | | | | |
| Ives | Catherine | Michigan State University | USA | Future of ABSP (Lewis) & Vit.A proposal (Chambers) | Washington, DC |

# TECHNICAL REPORTS & 2000 WORK PLANS

# ABSP Technology Transfer Activities

**Karim M. Maredia, ABSP**

## Transfer and Field Testing of Transgenic Potatoes

1. **South Africa:** The Agriculture Research Council in South Africa made an official request for B.t. potato lines from ABSP. A material transfer agreement has been developed and sent to South Africa for approval and signatures. ABSP will comply all the biosafety requirements of South Africa and USAID.

2. **CIP/Peru:** The International Potato Center (CIP) in Lima, Peru made an official request for B.t. potato lines from ABSP. B.t. potato lines were transferred to CIP in the form of tissue culture plantlets under an appropriate material transfer agreement. ABSP complied with all the biosafety requirements of the CIP and Peruvian government. ABSP will also work with the Peruvian hg0overnment and the USAID Biosafety Committee to obtain their approval before any field-testing is done in Peru.

3. **Egypt**: The Agriculture Genetic Engineering Research Institute (AGERI) has made a request for testing new B.t. Potato lines by ABSP. These lines will be transferred to AGERI in January 2000. ABSP will meet all the biosafety requirements of the Egyptian government and the USAID.

## Intellectual Property Rights (IPR) and Technology Transfer Training Activities

1. **IPR and Technology Transfer Internship Program:** Michigan State University in collaboration with the Institute of International Agriculture and ABSP organized a one week internship program in intellectual property rights and technology transfer from July 18 – 24, 1999. Twelve international participants from Colombia, Egypt, Ethiopia, India, Indonesia, Philippines, South Africa, Spain, Sri Lanka and USA attended this internship program. The ABSP Project sponsored participation of Dr. Tilahun Zeweldu from Ethiopia, Dr. Sugiono Moeljopawiro from Indonesia, and Cobus Coetzee from South Africa. The internship provided hands on experience in various aspects of IPR and technology transfer including basics of intellectual property rights, patents, copyrights, trademarks, plant variety protection, and trade secrets. In addition participants also learned about licensing and marketing of intellectual properties. The emphasis was on the day to day handling and management of intellectual properties.

2. **Food Safety Short Course:** The National Food Safety and Toxicology Center in collaboration with The Institute of International Agriculture at MSU organized a one week course on Food Safety from July 11 – 16, 1999. Twenty-five international participants from Albania, Egypt, El-Salvador, Estonia, Indonesia, Latvia, Mali, Saudi Arabia, Bulgaria and USA attended this course. The ABSP project sponsored participation of Dr. Taymour Nasr-El Din, and Mr. Hisham El-Sheshtawy from Egypt, and Dr. Muhammad Herman and Dr. Achmad Hidayat from Indonesia. The course focused on food safety policy development, risk analysis, and program implementation to ensure a safe food supply for the global community.

**Participation in Professional Meetings**

1. **AUTM Annual Meeting, San Diego, California:** Dr. Karim Maredia and Dr. Frederic Erbisch attended the annual meeting of the Association of University Technology Managers (AUTM) in San Diego, California from March 2 – 6, 1999. They participated in a session dealing with intellectual property on an international scene.

2. **National Biotechnology Meeting, Indonesia:** Dr. Karim Maredia and Dr. Frederic Erbisch attended the National seminar and exposition on research results of agricultural biotechnology from August 31 – September 1, 1999. They presented a paper entitled "Intellectual Property Rights and Commercialization of Agricultural Biotechnology products. Over 500 scientists, administrators, and policy makers from various institutions in Indonesia attended this seminar. During this visit to Indonesia, Drs. Erbisch and Maredia also visited the newly established office of intellectual property and technology transfer in Bogor and advised on the day to day operation of the office.

3. **International Biotechnology Conference, Washington, D.C:** Dr. Karim Maredia and Dr. Andrea Johanson attended the international conference on biotechnology at the World Bank from October 21 – 22, 1999. The conference covered the biotechnology and its potential impacts on agriculture in developing countries. Over 400 participants attended this conference.

## Publications

**K.M. Maredia, F.H. Erbisch, C.L. Ives, and A.J. Fischer**. 1999. Technology transfer and licensing of agricultural biotechnology in the international arena. AgBiotechNet., Vol. 1 May 1999. ABN 017.

**C.L. Ives, K.M. Maredia, and F.H Erbisch**. 1999. International Collaboration: Intellectual property management and partner country perspectives. In Managing Agriculture Biotechnology, Edited by Joel I. Cohen, CABI Biotechnology in Agriculture Series, No. 23.

# Commercialization/Technology Transfer/ Institutional Development -- AGERI, Egypt

## Introduction

This report outlines the activities and the achievements of AGERI during the period of October, 1999 through December, 1999 in the area of Commercialization/Technology Transfer/ Institutional Development.

The objectives of these activities within CUB project are:

- Establishment of a Pilot Technology Transfer Office
- Establishment of commercial linkages
- Institutional Development

## Achievements against objectives October-December, 1999

### Problem and Rational

Technology commercialization is the core of a new cost recovery policy of the Government of Egypt, which is part of an overall privatization policy to enhance the private sector role in the technology development. For AGERI and ARC, the implementation of such policy required:

1. Setting up an institutional framework to handle and manage proprietary technologies at AGERI
2. Issuing an internal acceptable IP policy, which specifies the right and duties of both the inventor and the institute regarding technology ownership, royalty and other issues of technology commercialization.
3. Running a comprehensive awareness program for all AGERI staff to cover all IPR issues (Technology, Management of technology, Licensing, Patenting, and other).

### Setting up an Office of Technology Transfer and Intellectual Property (OTTIP)

- ❑ A Head of the Office have been assigned (Dr Eid M A Megeed, a biotechnology transfer expert)

- Temporary office space has been allocated within the main building of AGERI (the permanent office will be located in the new building cross from AGERI).
- The office has been connected to the AGERI-LAN and Internet.
- Technology transfer and IP internal policy for AGERI have been drafted (in Arabic and English) by the Head of the office and discussed extensively with a committee from AGERI managers. The policy has been adapted officially from AGERI.
- A set of supporting documents have prepared to facilitate the office activities e.g., model form of licensing agreement, model form of Material Transfer Agreement, Model for of IP disclosure, a model form of Confidentiality disclosure, and model form of Profit Sharing Agreement. All the above documents have been prepared in both Arabic and English.
- One participant for AGERI attended the AUTM training course during the period July 15 until August 15, 1999.

# Development of virus resistant cucurbit crops using molecular genetic and conventional breeding approaches.


## Principal Investigators

Rebecca Grumet, Michigan State University

## Project partners

Molly Kyle Jahn, Cornell University
Dr. Atef Sadik, AGERI, Egypt
Dr. Hamdy El-Doweny, Horticultural Research Institute, Egypt


## Overall project goal

Our project goal is to develop virus resistant cucurbit crops using a combination of molecular genetic and conventional breeding approaches. We seek to develop novel transformation systems for cucurbit crops (R. Grumet) and couple the transformation capacity with ongoing breeding efforts (M. Jahn) to develop high quality cucurbits with multiple virus and disease resistances.

## Importance of the problem and rational for approach

Cucurbit species include a variety of high value crops (e.g., melons, watermelon, cucumber, summer squashes, winter squashes) that play important roles in both local diets and as export crops throughout the world. Currently a major limitation of successful production of these crops is infection by several viruses including the potyviruses, zucchini yellow mosaic virus (ZYMV), watermelon mosaic virus (WMV), the watermelon strain of papaya ringspot virus (PRSV-W), and the cucumovirus, cucumber mosaic virus (CMV). Crop losses of 50-100% in individual locations have been reported frequently.

During the past several years various groups, both commercial and public (including our group), have shown that it is possible to genetically engineer resistance to these viruses in cucurbit crops. In one case, virus resistant squash, originally released by the Asgrow Seed Company, has been produced commercially in the U.S. A major limitation to more widespread application of this technology to various cucurbit crops, is the lack of efficient transformation systems. For several species there are no available transformation systems; for other species the transformation systems can be very inefficient and/or highly genotype specific. Often the difficulty in developing effective transformation systems lies in the tissue culture based process that requires successful regeneration from individual cells. In the past few years, new, non-regeneration dependent methods of plant transformation have been developed for a small number of species. The primary motivating factors to develop such methods have been to bypass difficult and low efficiency regeneration protocols.

A major objective of this project is to develop a novel, non-regeneration based system for cucurbit transformation. We are working to adapt the electrotransformation system that was recently developed by Dr. Richard Allison (Michigan State University) for use with legume crops. Both

soybean and cowpea have been transformed using this method: approximately 10% of the treated plants subsequently produce transgenic progeny. If successful, this methodology would have value for any future traits to be incorporated, would have the added benefit of being broadly applicable across genotypes and even species, should be readily replicated in other laboratories, and would avoid the time, effort, expense and sophistication necessary for regeneration based systems. These features should make transformation technology more readily transferrable to developing countries. Because of interest expressed by collaborating ABSP countries, Egypt and Indonesia, we have initiated our efforts with cucumber. If successful, we will proceed to watermelon because of its economic importance and the lack of published transformation systems for this crop.

## Previous research

In the first phase of the project, the biotechnology research focused on the development of zucchini yellow mosaic virus (ZYMV) resistant melons. Key activities included development of a regeneration and transformation system using melon leaf explants, production of transgenic melons via *Agrobacterium* mediated transformation of cotyledon or leaf explants, verification of transformation and gene expression, and virus screening of transgenic lines in greenhouse and field trials (Fang and Grumet, 1993; Grumet et al. 1995; Yadav et al. 1996). The mleon transformation technology and the ZYMV coat protein construct were transferred to AGERI (Giza, Egypt) for use in melon and squash transformation. AGERI scientists produced transgenic squash lines showing resistance to ZYMV in the greenhouse and field.

In the current phase of the project our attention is focused primarily on the development of a non-regeneration dependent transformation system as described above. Our initial goals were to determine the parameters appropriate for electrotransformation of cucumber, including appropriate stage of seedling development, handling procedures before, during, and after electrotreatment to ensure transformation and recovery, and the appropriate electrotransformation settings for cucumber. Using ethidium bromide stained DNA to monitor entry of DNA into the cucumber tissue we were successful in modifying the original soybean-based procedure to adapt the DNA delivery system. We obtain 90 - 100% survival of the electrotreated plants.

Once these conditions were established, we treated approximately 200 plants and transferred them to the greenhouse for growth and fruit production. Cucumber plants subjected to the transformation procedure did not exhibit any apparent damage as compared to the non-treated control plants. When vegetative tissue of the treated plants was sampled by PCR analysis, 23 individuals (ca. 10%), indicated a positive response for incorporation of the introduced marker gene. This suggested stable integration of the gene that could be observed in upper leaves formed weeks after the initial treatment. No amplification product was observed in samples derived from control, non-treated plants. This frequency is very promising and is consistent with the results that have been obtained from soybean.

The electrotreated plants were hand pollinated to produce seed in the greenhouse. Fruit were harvested from approximately 150 plants. Seed were extracted from the harvested fruit and screened for transfer of the introduced gene to the next generation. Results of screening seeds from ca. 80 fruit did not yield PCR-positive individuals.

One possible explanation for the discrepancy between results from the originally treated plants and their progeny, is that the original treated plants are chimeric, i.e., they contain a mixture of transformed and non-transformed tissue. Given the nature of the original electrotreatment, it is reasonable to assume that only portions of the plant, and not the whole plant will become transformed. Since in the greenhouse we are only able to set 1-3 fruits per plant, when producing progeny we are not able to sample the whole plant. This can be contrasted with the situation with soybean where small numbers of seeds are set throughout the whole plant, rather than large numbers localized in one or a few fruits. This is a potential problem for cucurbits that we anticipated from the outset, and so we considered the possibility of modifying the approach if the initial method did not yield transgenic progeny. Modifications tested included introduction of a selection step to reduce chimerism of regenerated plants, and electrotransformation of floral meristems as described below.

## Research progress January – December, 2000

### *Electrotransformation*

Our first objective for 1999 was to build two apparatuses to allow electrotransformation to be performed in the Grumet lab (rather than doing the work in the R. Allison laboratory). Electrotransformation settings were re-optimized with the new equipment with emphasis on DNA concentration, insert size, vacuum infiltration, delivery time, and tip dimensions. We also have obtained the GFP reporter gene to allow for non-destructive determination of transgenic sectors to allow for directed pollinations in the greenhouse, and the BAR to allow for rapid selection of progeny. Transformation has been initiated with these reporters in addition to the NPT gene for kanamycin resistance.

To try to minimize the chimeric nature of the electrotreated plants, we tested inclusion of a selection step on kanamycin containing medium. Conditions for selection of excised apices were established and tested on ca. 100 electrotreated explants. Although we could select against non-transformed sectors, we did not observe growth of transgenic sectors.

At the time that we were performing these experiments, further analysis of the soybean system indicated that when transgenic progeny were obtained, in each case it was from the first pod to set seed (Allison et al., unpublished). This led to a revised view of the electrotransformation procedure suggesting that the gene introduction that leads to successful production of transgenic progeny may be directly incorporated into the developing floral primordium that is present at the time of electrotransformation. Given this information, we have revised the treatment protocols to treat older seedlings at a time when they would be in the process of initiating floral primordia.

In order to set fruit at the first nodes, we also switched to the use of the gynoecious pickling cucumber breeding line GY14. Future experiments will test whether it is important to have gene incorporation into developing ovules or developing pollen. Using the revised method approximately 160 treated plants have been successfully transferred to the greenhouse to produce progeny. Fruits have been harvested from ca. 50 plants. Additional fruits are currently developing in the greenhouse. We are now ready to initiate screening of the first sets of seed.

### *Agrobacterium-mediated transformation*

At the same time that we are actively working to develop an electrotransformation system for cucumber and other cucurbit species, we have also begun to revisit *Agrobacterium* based systems for cucumber, and have had very encouraging results. Cucumber

transformation has been very recalcitrant; in the published transformation reports, regeneration had been via somatic embryogenesis (Chee, 1990; Hammar and Grumet, 1990; Chee and Slightom, 1992; Sarmento et al. 1992; Schultz et al. 1995; Nishibashi et al. 1996; Rajharo et al. 1996). These methods were limited by low efficiency, extended time in tissue culture, and lack of reproducibility among laboratories.

This summer we tested a new method developed by Tabei et al. (1998) for a Japanese genotype and have been able to obtain reproducibly high regeneration rates with the American pickling cucumber, Straight 8. This system has the advantage of regeneration via organogenesis, which like the methods for melon, is more efficient and requires less time in tissue culture. The time in tissue culture to produce visible shoots is approximately 3 weeks, by 4-5 weeks shoots can be transferred to elongation medium. We have made modifications in the protocol to reduce vitrification of the regenerated shoots. We currently have ca. 40 regenerated cucumber plants in the growth chamber.

## Specific project objectives and work plan for 2000

Because of the potential advantages associated with the electrotransformation procedure, our efforts for 2000 will continue to put a strong emphasis on electrotransformation. We will, however, also continue to develop the *Agrobacterium* mediated method for cucumber transformation, including testing with genotypes of importance for Egypt and Indonesia. Our specific goals are:

1. Screen cucumber seeds that have been produced by the electrotransformation procedure. Verify putative transgenics by PCR and Southern blot analysis.
2. Monitor location of fruit producing transgenic seeds to optimize system for future efforts.
3. Continue to test factors that may influence DNA incorporation (plasmid size, cutting DNA).
4. Test whether the electrotreated plants can serve as pollen donors.
5. Test the *Agrobacterium* mediated organogenesis method with other genotypes, including Beit Alpha cucumbers and genotypes of importance in Egypt and Indonesia, to determine whether it is broadly applicable.
6. Set conditions to use the organogenesis method for transformation.
7. Produce transgenic cucumber plants and verify by NPT-ELISA, PCR, and Southern analysis.

## Publications citing ABSP support during the reporting period

Grumet R, Kabelka E, McQueen S, Wai T, Humphrey R. 2000. Characterization of sources of resistance to the watermelon strain of papaya ringspot virus in cucumber: allelism and co-segregation with other potyvirus resistances. Theor. Appl. Genet. In press.

Papadopoulou E, Grumet R. Transformation of melon (*Cucumis melo* L.). *In* The Handbook of Transgenic Food Plants. Hui YH (ed). Marcel Decker Inc. In press.

## Literature Cited

Chee PP. 1990. Transformation of *Cucumis sativus* tissue by *Agrobacterium tumefaciens* and the regeneration of transformed plants. Plant Cell Rep. 9:245-248.

Chee PP, Slightom JL. 1992. Transformation of cucumber tissues by microprojectile bombardment: identification of plants containing functional and non-functional transferred genes. Gene 118:255-260.

Fang G, Grumet R. 1993. Genetic engineering of potyvirus resistance using constructs derived from the zucchini yellow mosaic virus coat protein gene. Mol. Plant Mic. Interact. 6:358-367.

Grumet R, Yadav RC, Akula G, Hammar S, Provvidenti R. 1995. Genetic engineering of virus resistance in cucurbit crops. Proceedings Cucurbitaceae '94. In: Evaluation and enhancement of cucurbit germplasm. Lester GE, Dunlap JR (eds). P. 17-22.

Hammar S, Grumet R. 1990. Regeneration and *Agrobacterium* mediated transformation of cucumber. HortScience 25:1070.

Nishibayshi S, Hayakawa T, Nakajima T, Suzuki M, Kancko H. 1996. CMV protection in transgenic cucumber plants with an introduced CMV-O CP gene. Theor. Appl. Genet. 93:672-678.

Raharjo SHT, Hernandez MO, Zhang YY, Punja ZK. 1996. Transformation of pickling cucumber with chitinase encoding genes using *Agrobacterium tumefaciens*. Plant Cell Rep. 15;591-596.

Sarmento GG, Alpert K, Tang FA, Punja ZK. 1992. Factors influencing *Agrobacterium tumefaciens* mediated transformation and expression of kanamycin resistance in pickling cucumber. Plant Cell Tiss Org Cult. 31:185-193.

Tabei Y, Kitade S, Nishizawa Y, Kikuchi N, Kayano T, Hibi T, Akutsu K. 1998. Transgenic cucumber plants harboring a rice chitinase gene exhibit enhanced resistance to gray mold (*Botrytis cinerea*). Plant Cell Rep. 17:159-164.

Yadav RC, Saleh MT, Grumet R. 1996. High frequency shoot regeneration from leaf explants of muskmelon. Plant Cell Tis. Org. Cult. 45:207-214.

# Development of Disease-Resistant Cucurbit Crops


## Principal Investigators

Molly Jahn, Cornell University

## Project partners

Rebecca Grumet, Michigan State University
Dr. Atef Sadik, AGERI, Egypt
Dr. Hamdy El-Doweny, Horticultural Research Institute, Egypt


## Project Goals

1. Use classical plant breeding approaches to improve cucurbit crops for resistance to ZYMV, and to develop genetic resources for resistance to three other cucurbit viruses of worldwide importance, cucumber mosaic virus (CMV), watermelon mosaic virus (WMV), and papaya ringspot virus (PRV).
2. Provide adapted varieties of squash, melon and cucumber with additional disease resistances for use directly as varieties, as advanced breeding lines for further breeding efforts in developing countries, and to provide multi-resistant genotypes for the transformation efforts.

## Rationale

Because of the worldwide importance of cucurbit crop losses to viral diseases, we have concentrated especially on building genetic resources for resistance to the major viruses in squash (*Cucurbita pepo*), melon (*Cucumis melo*) and cucumber (*C. sativus*). Our goal first was to locate genetic resistance from wild accessions within or related to each of the cultivated species to cucumber mosaic virus (CMV), papaya ringspot virus (PRV), watermelon mosaic virus (WMV) and zucchini yellow mosaic virus (ZYMV). Once we found sources of resistance to each of these diseases, we have hybridized these genotypes with cultivated types that lack resistance but possess many desirable attributes. We have emphasized the use of types that are important in Egypt, and elsewhere in the Middle East, North Africa, and Asia. We work to transfer each resistance so that eventually we will convert the cultivated types to multiresistant versions. In addition to the four viral diseases, we also work with resistance to important fungal diseases, and in some cases, also to insects. In cucumber, we have germplasm with resistance to the major leafspots including *Alternaria*, anthracnose, *Cladosporium*, *Ulocladium*, bacterial wilt, downy and powdery mildew: in melons, downy and powdery mildew, gummy stem blight; and in squash, powdery mildew and black rot.

We have provided our material for field testing in Egypt, and with the support of Phase II of this project, to other locations including Jordan, Turkey, the Philippines, Indonesia, India, and Morocco. We work with seed companies and public sector breeding and testing programs where we can find suitable partners.

# Current Research

## Cucumber

In cucumber, resistance to the four viruses is conditioned by recessive genes that have been combined in U.S. slicer types which served as a donor for the Phase I program that concentrated on the Middle Eastern cucumber, Beit Alpha. Beit Alpha has been essentially converted to carry all four virus resistances and some leafspots. In a recent field trial conducted by Dr. Henry Munger in Ithaca, NY, this material yielded at least comparably and held longer in the field than an extremely popular commercial variety in the Middle East, Peto's

## Cucurbita spp.

In squash, resistance to the four viruses in *Cucurbita pepo* has been obtained by interspecific crosses from wild types of *C. martinezii* (CMV), *C. moschata* (CMV, PRV, WMV, and ZYMV), and *C. ecuadorensis* (ZYMV). Currently we are combining resistance to CMV and ZYMV with powdery mildew resistance (PMR) in zucchini types which serve as donor lines for the Eskandarany program. As part of the Phase I effort, we re-examined the *C. moschata* resistance source, 'Nigerian Local', and have discovered that the high levels of CMV, PRV and WMV resistance are conferred by single genes. These resistances were transferred interspecifically and resulting *C. pepo* breeding material has been successfully trialed in Egypt as a part of Phase I. These materials were still very rough for horticultural type, but the resistance held well in our own tests and field trials in Ithaca, NY and in commercial trials conducted elsewhere in North America and the Middle East.

In 1999, we conducted a spring greenhouse generation to advance *C. pepo* and *C. moschata* breeding materials harvested from the field in 1998. We harvested seed from that generation, and seeded our field planting on May 26, 1999. 1999 was a year of unprecedented drought stress for us, nevertheless we were able to maintain plants with controlled pollinations til harvest in almost all cases. The squash virus planting consisting of 840 F2 & F3 plants was inoculated twice with a virus cocktail containing equal parts CMV, ZYMV, PRSV and WMV. The first inoculation was at the cotyledon stage the second at the first true leaf stage. All plants were transplanted to the field and observed for disease development. Tolerant / resistant plants were self-pollinated, sib-pollinated, or crossed to Eskandarany. Data was also taken on powdery mildew resistance. From this field, 65 fruit from controlled pollinations were harvested from the most resistant plants and are being tested for all four viruses separately in the greenhouse now.

For this winter, 2000 screen, 12 plants of each line were inoculated with each of the following three viruses, CMV, WMV, ZYMV and 24 seedlings per line were inoculated with PRSV because of its recessive inheritance. To date we have not found a line that is homozygous resistant to all four viruses, however we have identified lines in bush types that are homozygous resistant to one or more viruses as follow:

**8 lines with plants resistant to one virus**
3 lines with CMVR
2 with WMVR
2 with ZMVR
1 with PRVR

**11 lines with plants resistant to two viruses**
5 with WMVR + ZYMVR
3 with CMVR + ZYMVR
1 with PRVR + WMVR

1 with PRVR + ZYMVR
1 with CMVR + WMVR

**5 lines with plants resistant to three viruses**
2 with PRVR + WMVR + ZYMVR
2 with CMVR + WMVR + ZYMVR
1 with CMVR + PRVR + ZYMVR

Only one line had plants classified as resistant to 4 viruses, but this line was not homozygous to any of the viruses. We are concentrating on pollinations that will combine virus resistances to all four viruses and improve plant and fruit types. All of the F1 progenies from a second backcross made in the field were uniformly susceptible, which does not follow Mendelian ratios and is probably a consequence of the interspecific nature of this cross. We will continue to use a pedigree approach to avoid this problem, therefore at this point, we are intercrossing, selfing and sibbing the highly resistant plants identified in this screen. This seed will be harvested, screened again, and then transplanted to the field during the summer, 2000 for evaluation for type and resistance to additional viruses.

## *C. moschata*

East West Seed Co. in the Phillipines has asked us to breed multiple virus resistance in 3 of their *C. moschata* varieties, Bugong, Batangas and Rizalina. This material was planted with a wide variety of early generation Nigerian Local material, the source of resistance to all four viruses. The East West material did not produce flowers until September making it difficult to get mature seed from any crosses, however we did achieve the following pollinations which yielded seed. These are large plants and we are currently unable to handle them in the greenhouse due to the fact that we have received only about half of the funds we have budgeted for. We have used the core funds we have received to support Egypt activities as well as activities for the rest of the developing world. When we receive the funding now 1 1/2 years later than we had planned, we should have sufficient funds to rent the greenhouse space necessary to support these activities for other parts of the world.

**Crosses made**

| | |
|---|---|
| 155 X 153 | ZMR BN X Bugong |
| 157 X 153 | (BN X NL)F1 X Bugong |
| 168 X 153 | (BN X NL)F3 X Bugong |
| 154A X 161 | Batangas X (BN X NL F2) X NL] |
| 154B X 155 | Rizalina X (BN X NL F1) |
| 154B X 157 | Rizalina X ZMR BN |

## Melon

In melons, we have resistances in various combinations in the cantaloupe variety, TAMUvalde, which is a high quality melon, very well adapted to warm conditions, that carries both PMR and DMR. This melon had been selected previously by Dr. El Doweny to be of interest for the Egyptian program. This melon will be useful directly and in combination with Egyptian types. We also have bred virus resistant versions of a classic cantaloupe variety, Topmark, a Western shipper type with good quality and storage characters. We have also developed multiple resistance in green crisp fleshed types such as Honeydew, which could be combined quite easily with the Galia type, becoming ever more popular around the world.

In early 1998, 25 Topmark progenies and 38 Honeydew progenies were harvested and turned over to the seed industry for increase and additional trialing in North America, Asia and the Middle East. These seed lots have been evaluated repeatedly with the three potyviruses but have their most recent backcrosses to the best CM-tolerant Topmarks and Honeydews we have. Resistance/tolerance to all four viruses should be found in this material. During spring, 1999 about 2000 plants were screened for ZYMV and CMV resistance (PRV and WMV tests were also performed with less reliable results) Survivors from all tests were planted together in a field evaluation.

During the summer of 1999 we also evaluated 60 progenies from all three backgrounds that had been returned from off season increases by commercial cooperators during the winter 1998-99. In the field evaluation the results showed that we have the best resistance in the Topmark and Honeydew backgrounds. Our CMV and ZYMV inoculation expressed the most reliable results. The PRV and WMV inoculation showed delayed expression of symptoms and by then, virus had moved through the field confounding evaluation.

| | |
|---|---|
| **Topmarks** | 272 plants 24% resistance for CMV |
| | 262 plants 23% resistance for ZYMV |
| **Honeydews** | 290 plants 28% resistance for CMV |
| | 293 plants 37% resistance for ZYMV |
| **TAMUvaldes** | 158 plants 18% resistance for CMV |
| | 166 plants 20% resistance for ZYMV |

We emphasized self-pollination of the most resistant individuals in all backgrounds. We also were provided some melons from the Philippines. Surprisingly the material from the Philippine's adapted very well to our short growing season but our attempts to cross with some of our virus tolerant material was hindered by severe mildew susceptibility.

In the fall of 1999 we planted the best progenies and selfs we were able to obtain from the summer field planting to be evaluated for multiple disease resistance. The test consisted of 12 Topmark, 33 Honeydew, 9 TAMUvalde and 3 ZPPM 399 progenies with about 3587 plants total.

| | |
|---|---|
| **TopMarks** | 189 plants 3% resistance for CMV |
| | 197 plants 21% resistance for ZYMV |
| | 258 plants 29% resistance for WMV |
| | 236 plants 14% resistance for PRV |
| **Honeydews** | 496 plants 9% resistance for CMV |
| | 488 plants 18% resistance for ZYMV |
| | 605 plants 18% resistance for WMV |
| | 596 plants 29% resistance for PRV |
| **TAMUvaldes** | 101 plants 10% resistance for CMV |
| | 85 plants 45% resistance for ZYMV |
| | 123 plants 30% resistance for WMV |
| | 113 plants 17% resistance for PRV |

Selections from this screen are being pollinated now. We are largely intercrossing (pedigree breeding) to stabilize and boost resistance levels. Intercrossing will also allow us to pyramid multiple resistances. This approach has been very successful in the squash breeding program.

## Cucumber

We screened 1445 cucumber plants with four viruses in the fall, 1999. They were planted and inoculated along with the melon material. The virus did not develop as fast on the cucumber and with later expression viral classification was made difficult. We are working on identifying a smooth (non-warty) fine spine uniform cucumber with resistant to all four viruses and the leafspots. In this test, a number of lines compared favorably to Indy (Peto), which claims to be tolerant to all four viruses.

## Implications of Research Findings and Commercialization

In early 2000, we signed the first licensing agreements for several items produced with some support from this project including multi-virus resistant cucumbers and melons, and squash resistant to powdery mildew. Generic drafts of these licensing agreements are available upon request. We have provided germplasm to companies operating all over the world and we have conducted major trials in Egypt (still waiting to hear how our materials performed), Jordan, Morocco and smaller trials in India, Indonesia and the Philippines. We now have strong partnerships with companies active in the Middle East, North Africa, and Asia, and hope to strengthen our ties with public sector breeders in these regions where they are active.

## Publications

Anagnostou, K., M.K. Jahn and R. Perl-Treves. 2000. Inheritance and linkage analysis of resistance to zucchini yellow mosaic virus, watermelon mosaic virus, papaya ringspot virus and powdery mildew resistance in *Cucumis melo* L. accepted Euphytica.

Zuniga, T., J.P. Jantz, T.A. Zitter and M.K. Jahn. 1999. Monogenic dominant resistance to gummy stem blight in two melon (*Cucumis melo* L.) accessions. Plant Dis. 83:1105-1107.

Silberstein, L., I. Kovalski, R. Huang, K. Anagnostou, M.K. Jahn and R. Perl-Treves. 1999 Molecular variation in melon (*Cucumis melo* L.) as revealed by RFLP and RAPD markers. Scientia Horticulturae 79:101-111.

## Workplan for 2000

Our original workplan has not changed and we are on schedule with all activities. We will continue our breeding efforts in all species in Ithaca, NY and in cooperation with seed companies with activities in the developing world. We will be releasing lines with resistance to one or more viruses combined with fungal resistances when available as we approach suitable type. We plan to attend the meetings in Egypt in May to exchange germplasm and results.

# Development of viruses resistant cucurbit crops using a combination of molecular genetics and conventional breeding approaches


## Principal Investigators

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Dr. Hamdy El-Doweny, HRI, Egypt

## Egyptian Research Team

| | |
|---|---|
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| | Hanan Faried |
| | Attia Omr |
| **Melon:** | Gihan Hosny |
| | Roba Ismail |
| | Tarek Tawfik |


## Overall Project Goal

The main goal of this project is to develop virus resistance in some cucurbit crops using a combination of molecular genetics and conventional breeding approaches. In Egypt, a number of experiments were designed to: 1) continue development of virus resistance in squash, cucumber and melon via genetic engineering approaches by introducing the coat protein gene(s) of ZYMV and/or CMV, 2) establish transformation and/or regeneration system in cucumber, 3) evaluate transgenic squash, cucumber and melon lines produced and 4) evaluate cucurbit material(s) developed by Cornell University under Egyptian field conditions.

## Importance of the problem and rationale for approach

Cucurbit species include a variety of high value crops (e.g., cucumber, melon, watermelon, squashes) that play important roles in local diets and as export crops. The area under cultivation with squash in Egypt is around 78,000 feddans and producing about 568,000 tons. In addition, the export values for melon and watermelon exceed $ 1 million annually ($ 749,000 and $ 310,000, respectively).

In Egypt, the cultivated cucurbits are infected by many diseases in common, causing reduction in yield and quality. These crops can be completely destroyed when infected by zucchini yellow mosaic potyvirus (ZYMV), cucumber mosaic cucumovirus (CMV) and/or watermelon mosaic potyvirus (WMV).

The control of such viruses using insecticides and/or inspection and rouging is ineffective. Therefore, cultivars resistant to such viruses would be the most effective means for its

control. Transgenic varieties that are virus resistant, based on virus-derived transgenes has been widely demonstrated to be an effective strategy for control of such viruses and could increase productivity and reduce inputs. In the past few years, protocols for regeneration and transformation have been developed for a small number of cucurbit crops. Under the ABSP/AGERI collaborative project we first initiated our efforts with squash followed by cucumber. If successful, we will proceed to watermelon because of its economic importance to Egypt and the lack of published regeneration and transformation systems for this crop.

## Previous Research Achievements

**Transformation and regeneration**

- We were successful in introducing the ZYMV-CT-CP gene into the Egyptian Eskandarani cultivar of squash *via Agrobacterium*-mediated gene transfer system using shoot tip explants and *Agrobacterium tumefaciens* LBA4404 strain harboring the pGA643 plasmid.
- Twenty two out of thirty $R_0$ kanamycin resistant squash lines were successfully acclimatized onto peat-moss:soil (2:1, v/v) under controlled environmental conditions in the Biocontainment facility at AGERI.
- PCR technique confirmed the presence of ZYMV-CT-CP gene in the 22 $R_o$ kanamycin resistant squash lines.
- Results of DAS-ELISA detection showed that the expression of ZYMV-CT-CP gene was found in 15 out of the 22 $R_o$ tested squash lines.

**Virus evaluation of $R_0$ transgenic plants**

- Lines 5, 6 and 17 were the most resistant followed by lines 2, 9, 11, 13 and 22, as mild symptoms appeared after 6 and 4 weeks respectively. On the other hand, symptoms were observed on the other lines as well as control after 2-3 weeks from ZYMV-E inoculation.
- The eight resistant lines were subjected to self pollination and six out of eight lines namely 5, 6, 9, 11, 17 and 22 were selected based on fruit quality and agronomic characteristics including production of good quality R1 seeds.

**Virus evaluation of $R_1$ transgenic plants**

- A number of about 24 of 212 transgenic squash plants (11.3%) were found to be resistant to ZYMV-E, while the non-transformed inoculated squash plants were deformed and completely destroyed after three weeks from virus inoculation.
- Lines 5 and 17 were most promising since there was a delay in developing ZYMV characteristic symptoms until the tenth week from virus inoculation. After self pollination 10, 7, 5, 10 and 1 fruits were produced from lines 5, 6, 9, 17 and 22, respectively.

**Virus evaluation of $R_2$ transgenic squash plants**

- Line 5 was found to be more resistant than line 17. Two plants from family H (line 17) gave no symptoms on the young leaves after ten weeks from virus inoculation. Nine good fruits belonging to families A (2), B (1), D (1), E (1), F (1) and H (3) were produced as a source for R3 seeds.

**Virus evaluation of $R_3$ transgenic squash plants**

- Families H1, H2 and H3 appeared to be highly resistant against ZYMV-E isolate.

- The majority of these plants gave no symptoms on the young leaves after eight weeks from virus inoculation.

## Publications

Said, M. Khalil, Atef S. Sadik, Hamdy El-Doweny and Magdy A. Madkour (1999). Production of transgenic squash plants resistant to zucchini yellow mosaic potyvirus. Arab Journal of Biotechnology, 2(1): 27-44.

## Specific Project Objectives

1. Continue the evaluation of highly ZYMV-tolerant squash lines ($R_4$) under open field condition (**5 months**).
2. Mass selection for two generations (**12 month**).
3. Evaluation of multiple virus resistant lines in Egypt (**6 months**).
4. Establishment of regeneration system in cucumber (**6 months**).
5. Establishment of transformation system in cucumber (**8 months**).
6. Introducing the coat protein gene(s) into some cultivars of squash, melon and cucumber (**8 months**).
7. Evaluation of $R_0$ transformed plants (**6 months**).
8. Evaluation of $R_1$ transformed plants (**6 months**).
9. Evaluation of $R_2$ transformed plants (**6 months**).

## Research Progress

### Objective 1: Continue the evaluation of highly ZYMV-tolerant squash lines ($R_4$) under open field conditions:

**First field trail (March, 1999)**
Seeds from each of families H12, H22 and H31 (line17) were planted at Sids Station, Bani Sweif Governorate (under the supervision of Dr. H.El-Doweny, HRI, ARC, Giza) in a replicated for evaluation trial as shown in Figs. 1 and 2. Non-transgenic squash of the same variety were also included to serve as control in addition to nineteen other commercial squash varieties. The virus resistance of these plants was evaluated based on symptomatology as mentioned before. Two replicates were inoculated twice with ZYMV-Egy, while the third was left without virus inoculation. Results showed that the majority of the evaluated plants appeared highly resistant (92 and 96 %, respectively) against ZYMV-Egy isolate, as no symptoms showed until the eighth week from virus inoculation. Only two families, H12 and H22, showed good marketable fruit characteristics and high yieldily ability as compared to the control.

**Second field trial (September, 1999)**
The same lines were re-evaluated for their virus resistance under field conditions, but in this experiment, the non-transgenic plants were only inoculated with ZYMV-Egy isolate to serve as a virus source. The selected transgenic lines were left with no virus challenge

and without any insecticide treatments. Furthermore, the neighboring plants, i.e., cucumber, squash, maize, weeds and cantaloupes were subjected to virus detection randomly to study the possibility of gene flow under field conditions. The results of this work will be included in the next report.

Permit No 22 was issued by the National Biosafety Committee to conduct these two field trials at Sids Experimental Station.

**Fig. 1. Virus evaluation of transgenic squash lines under field conditions in Sids Station.**


**Fig. 2. Squash fruits produced from transgenic ZYMV-resistant lines. A, good marketable fruits produced under field conditions (Sids Station). B, fruits harvested from the two selected families for seed production.**

## Objective 3: Evaluation of multiple virus resistant lines in Egypt

Unfortunately, we did not receive any multiple virus resistant lines form Cornell University to be subjected for virus evaluation under the Egyptian field conditions as planned in the first year of the program of this project.

## Objective 4: Establishment of a regeneration system in cucumber

Mature cotyledons of cucumber (*Cucumis sativus* cv. Beit Alpha MR) was used as a source for explants in the regeneration trials *via* organogenesis, while cotyledonary leaves were used for regeneration *via* embryogenesis. MS medium supplemented with different concentrations of hormones was used for establishing the system.

### Results

- Combining 2,4-D with kinetin showed significant effect on callus induction from cotyledonary leaves. The highest level of embryonic callus production was obtained on MS medium supplemented with 1.0 mg/l 2,4-D and 0.5 mg/l kinetin. Subculturing such calli on MS with 0.5 mg/l kinetin and 1.0 mg/l NAA proved to give best results for production of plantlets *via* somatic embryogenesis.
- Regeneration *via* organogenesis was achieved when mature cotyledon explants were cultured on MS medium supplemented with 2.0 mg/l BAP and 0.3 mg/l NAA.
- Root formation was achieved when shoots, produced from mature cotyledon explants, were cultured on MS with 0.1 mg/l NAA.
- The use of plastic bags for 10 days while regenerated plants were being acclimatized increased the number of surviving plants. Soil mixture containing peat-moss and clay at a ratio of 1:1 was the best for acclimatization and production of mature regenerated plants.
- These results are illustrated in Figures 3,4 and 5.

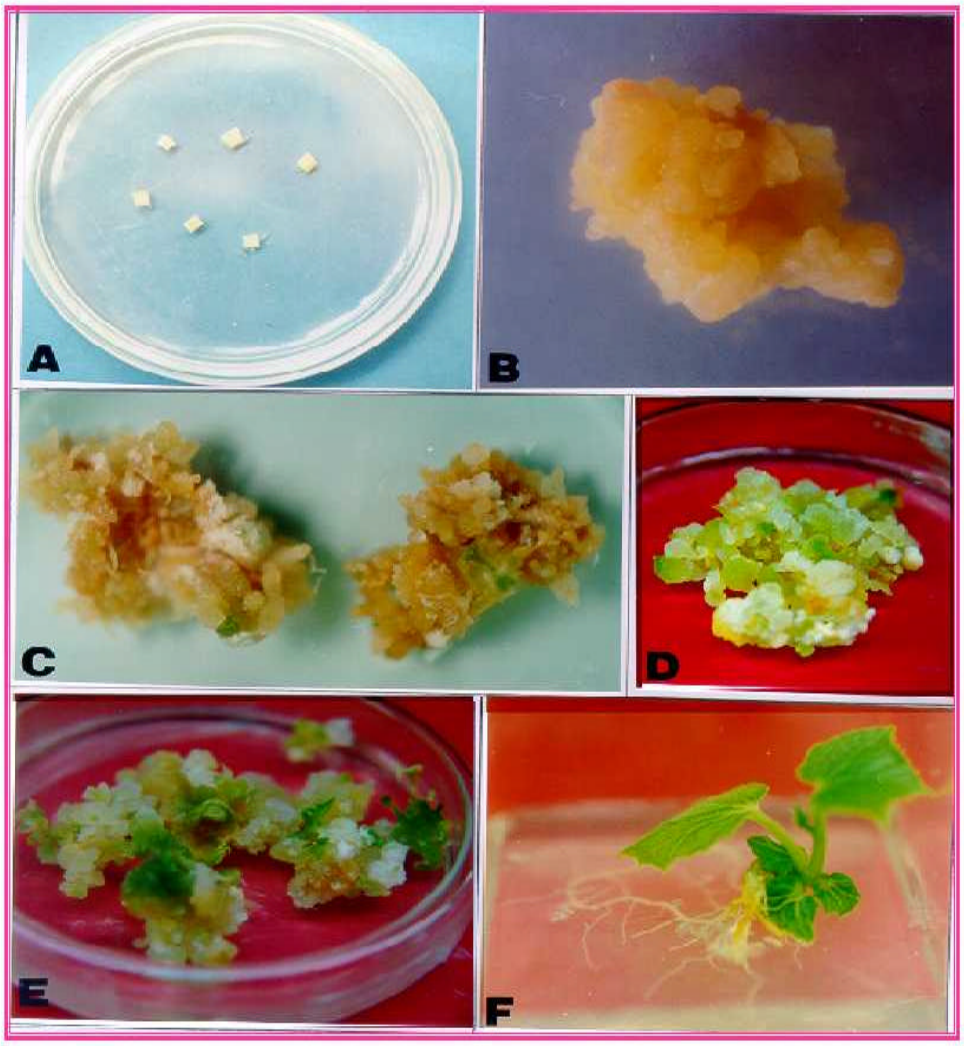


**Fig. 3. Somatic embryogenesis and production of plantlets from cotyledonary leaf explants of cucumber (*Cucumis sativus* L. Beit Alpha MR). A, cotyledonary leaf explants on induction medium. B, callus formation on induction medium; C, embryonic callus showing early multicelluar stages of embryos on maturation medium MS supplemented with 1.0 mg/l NAA and 0.5 mg/l Kinetin); D, differentiation of embryo on maturation medium; E, maturation of the somatic embryos on a hormone free MS medium and F, germination of embryo and production of plantlet.**

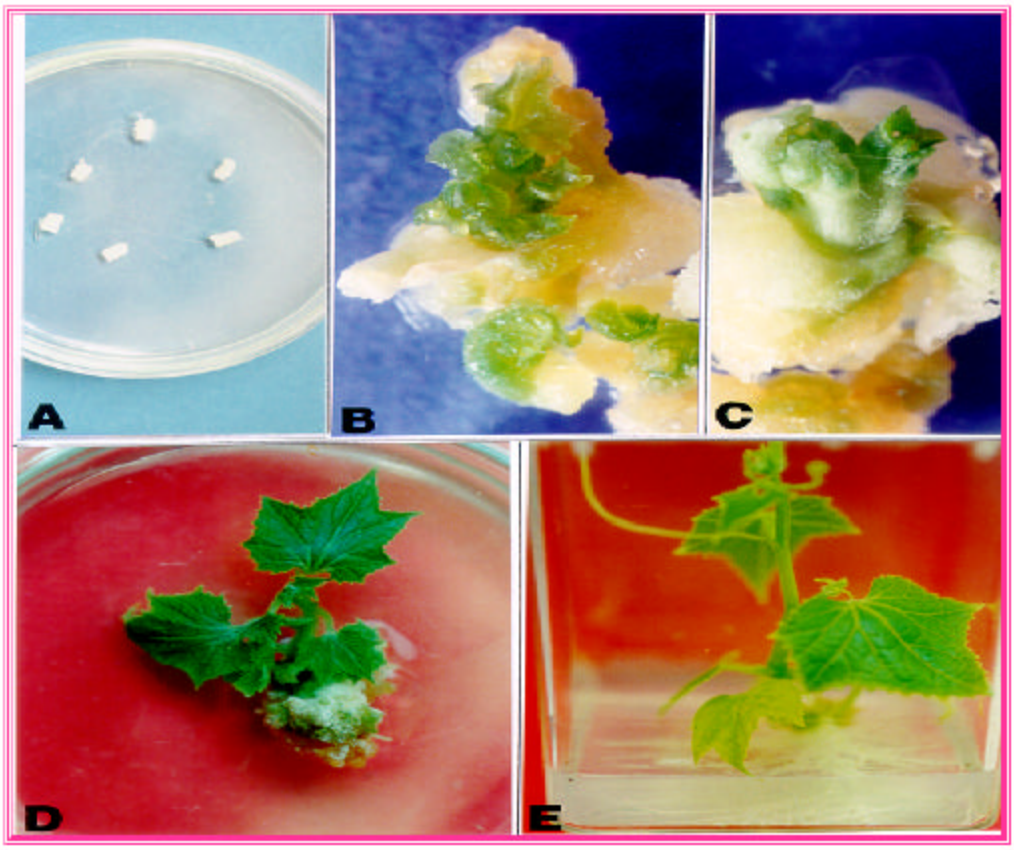


Fig. 4 **Regeneration and production of plantlets *via* organogenesis from mature cotyledon explants in cucumber on an MS medium supplemented with 2 mg/l BAP and 0.3 mg/l NAA.** A, **Mature cotyledon explants;** B, **callus formation and development of shoot primordia.** C, **developmental shoot;** D, **elongation of shoots, 4-5 weeks post plantation on a regeneration medium. and** E**, root formation and production of a plantlet on MS medium supplemented with mg/l NAA.**

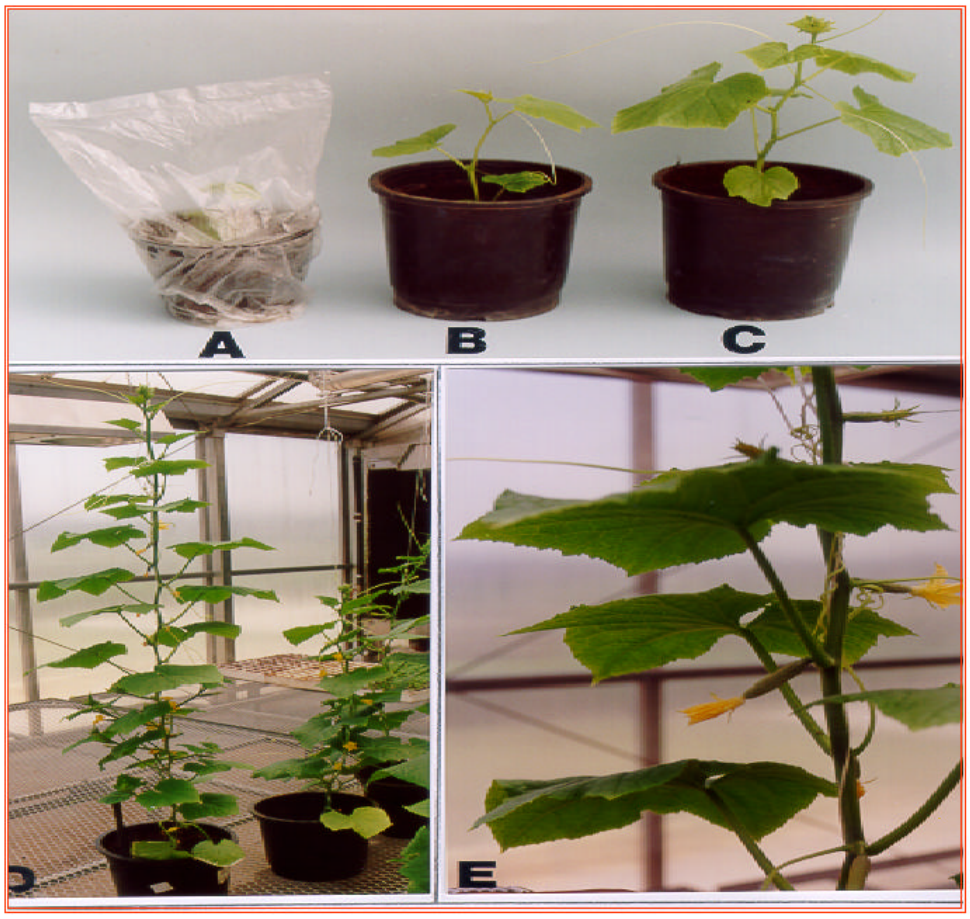


Fig. 5. **Acclimatization stages of regenerated cucumber plantlets using a soil mixture containing peat-moss : clay (1:1, v/v);** A**, plantlet 3 days post plantation using plastic bag covering;** B**, a regenerated cucumber plant 2 weeks post plantation;** C**, a well developed cucumber plant, 3 weeks post plantation;** D**, mature regenerated cucumber plants producing male and female flowers 6 weeks post plantation and** E**, normal female flowers.**

## Objective 5: Establishment of transformation system in cucumber

Transformation system was established using pBI121 plasmid containing GUS and NPT-II reporter and selectable marker genes, respectively (Fig. 6). Putatively transformed plants were selected on a regeneration medium supplemented with 100 mg kanamycin/l (Fig. 7 ). Successful transformation was then confirmed using PCR and Southern blot hybridization techniques.

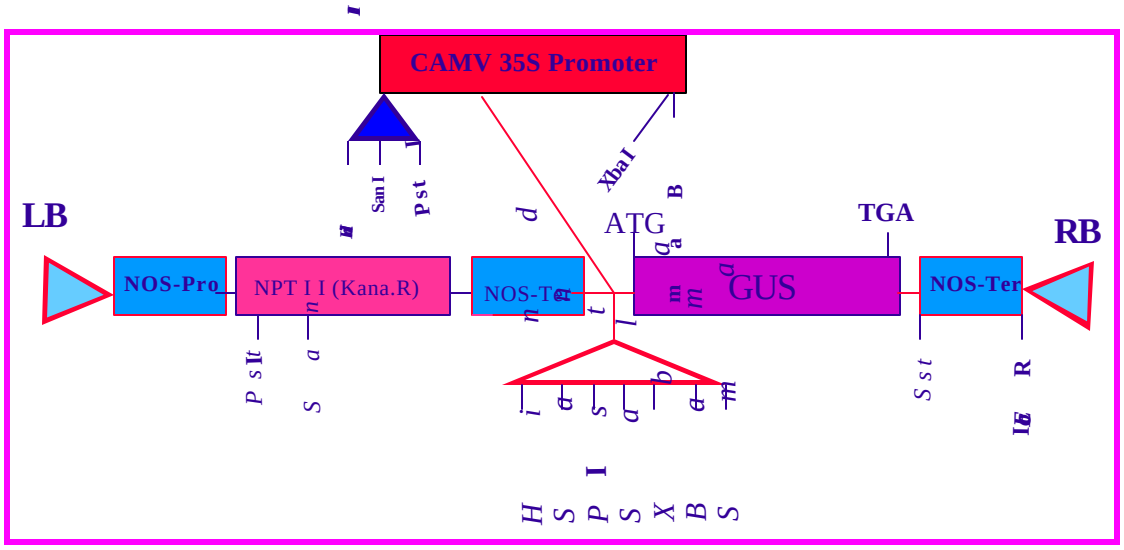


Fig. 6. **Restriction map of the pBI121 plasmid containing NPT II gene with NOS promoter and terminator and GUS gene with cauliflower mosaic virus 35S promoter and terminator** .

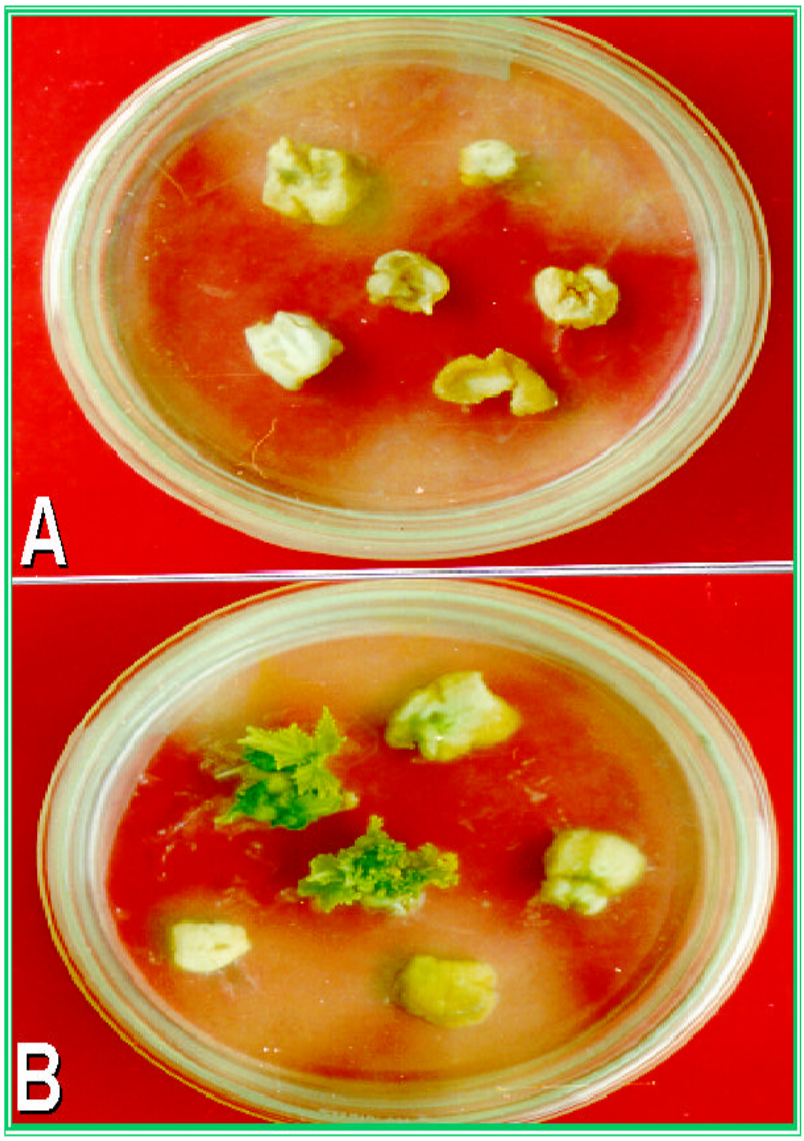


Fig. 7 **Selection and growth of putative transgenic shoots of cucumber. Mature cotyledon explant of cucumber cultured on regeneration medium supplemented with 2 mg/ BAP, 0.3 mg / NAA, 100 mg/l kanamycin and 400 mg/l carbincillin.** A, **inoculated explants with non-transformed *A. tumefaciens* strain LBA4404;** B, **Explants inoculated with *A. tumefaciens* LBA4404 + pBI121 recombinant plasmid containing GUS + NPT-II.**

## Highlights of significant achievements

- Establishment of regeneration and transformation system in the Beit Alpha MR open field cultivar of cucumber.
- Evaluation of $R_4$ transgenic squash lines under field conditions and selecting high yielding ZYMV resistant lines with good marketable quality fruits.

## Plan of work for the year 2000

1. Isolation of the coat protein gene of cucumber mosaic *cucumovirus* (CMV-Egy) followed by sequencing, cloning and constructing a cassette to be ready for introducing into some cucurbit crops.
2. Introducing the coat protein gene(s) into cucumber, melon and squash cultivars.
3. Putatively transgenic plants will be tested for the incorporation of the introduced genes by ELISA -using ELISA kits specific for virus(s) of interest and also PCR analysis.
4. Progenies will be screened for the presence of the introduced genes by PCR and Southern blot analysis followed by evaluation of virus resistance in the produced transgenic plants under greenhouse and field conditions.

# Potato Transformation for Development of Potato Tuber Moth Resistance

## Principal Investigators

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## Project Partners

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## Account Number

61-2899

## Overall Project Goal

The overall objective of the project is to test resistance management theories with respect to maintaining effectiveness of host plant resistance in potato against potato tuber moth. The specific objectives are to:

- Continue genetic engineering development with emphasis on genes available for commercial development.
- Continue transformations with new gene/vectors for varieties important to Egypt.
- Examine the individual foliar expression levels of new transformations in lab and field tests.
- Evaluate the effectiveness of individual and combined resistance factors under laboratory experiments.
- Develop linkages with US companies and Egyptian seed companies to promote the commercialization of desired potato lines.
- Evaluate different management strategies for maintenance of resistant potato varieties and their integration into IPM systems.

## Importance of the Problem

Potato is one of the most important vegetable crops in Egypt with total production up to 2 million tons annually. It is also the leading export vegetable in Egypt, with approximately 225,000 tons exported to the United Kingdom and western European countries. Potato is cultivated in the Nile delta region on about 34,000 hectares. Most of the exported potato

production is centered in Behira, Menofya and Garbiya, where yields in these three governates range between 42.5 and 58.5 tons/hectare (Hasan 1991).

The primary insect pest in Egyptian potato production, like many other countries in the Middle East, is the potato tuber moth, *Pthorimaea operculella* (Zeller). In the field, the moths lay their eggs on the potato foliage and the hatched larvae mine the foliage and the stems. This feeding damage leads to irregular transparent tunnels in the leaves and weakening of the stem. The larvae attack the tubers through infected stems or directly from eggs, which are oviposited on exposed tubers or where soil cracks allow moths to reach the tubers. Larvae mine the tuber in the field and in storage reducing potato quality and increasing the potential for pathogen infection. In Sudan, about 30 – 40 % of the potatoes are stored in underground pits and can be completely destroyed by tuber moth within two months (Ali 1993).

## Project Background

This project was started in 1992. Initially, training was a key component of the project. Scientists from AGERI visited MSU to learn techniques about vector construction, potato transformation, field testing of transgenic potatoes, potato tuber moth bioassays, intellectual property rights and biosafety.

## Rational for Approach

The CryV Bt toxin gene has been codon modified to increase its expression level in the plant and transferred into potato. Douches et al. (1998) used different CryV constructs for engineering the potato to express high level of Bt toxin effective against potato tuber moth. Some of these transgenic lines were evaluated to determine the foliar resistance to potato tuber moth (Westedt et al. 1998). Li et al. (1999) produced a series of CryV-Bt and transformed the cultivar Spunta. Foliage bioassays with potato tuber moth revealed high expression levels. For these transgenic lines to be commercially successful in reducing potato tuber moth damage, we must assess the tuber resistance from the field and storage. The objectives of this study are to evaluate field-grown CryV-Bt transgenic potato tubers for their resistance to potato tuber moth. In addition, to evaluate of 11-12 month old stored transgenic tubers to determine the mortality efficiency of the expressed CryV-Bt toxin.

In most of Egypt, at least on smaller farms, storage of tubers is in non-refrigerated nawallas, which leads to most of the insect damage and to high levels of insecticide spraying of tubers in storage. These transgenic potatoes will therefore reduce the use of insecticides in Egypt thus allowing for the production of a safer product for human consumption. Also, potato production with less insecticide use will have less impact on the insecticide contamination of the environment.

## Previous Research

Initially, transformations with the Cryla(c) wild type gene were performed using cv. 'FL1607' as a model system (Hudy 1997). Yadav and Sticklen (1995) developed a genotype independent potato leaf disk regeneration protocol. This regeneration protocol was adapted to our Agrobacterium mediated transformation protocol (Douches et al. 1998). The first Cry5-Bt construct (with the GUS gene fused to the Cry5-Bt gene) was used in transformations with cvs. 'Lemhi Russet', 'Atlantic', L235 - (glandular trichome line), and USDA838~1 (foliar leptine line) (Westedt et al. 1998). The CryV-Bt constructs that differ in the promoter (CaMV 35S, Gelvin super promoter and patatin promoter) were transformed into cv. 'Spunta' (Li et al. 1998a). The Cry5-PVYcp gene construct was also transformed into 'Spunta' (Li et al. 1998b). Spunta is the most important cultivar grown in

Egypt, while Atlantic is a desired chip-processing cultivar. Other constructs that have the GUS gene removed are ready to use in transformation. A sample of the Cryl and Cry5-t transgenic lines was transferred to AGERI as tissue culture plantlets for greenhouse testing.

Detached leaf bioassays are used to determine the level of host plant resistance to potato tuber moth. Various potato lines were screened for natural resistance to potato tuber moth. All PCR-positive Bt transgenic lines developed from this project were screened for resistance to PTM. In addition, a series of other transgenes were evaluated but had no effect upon PTM mortality. We also obtained a number of synthetic Cryla-transgenic potato lines from the USDA to test; these lines gave strong control of the tuber. The most promising lines from the detached leaf tests were also advanced to laboratory tuber bioassays. Tuber bioassays identified a series of Cry5-Bt-Spunta and Cry5-Bt/PVY-Spunta with high levels of potato tuber moth mortality (Li et al 1998a). Other Cry5-Bt-transgenic lines (Atlantic, Lemhi Russet and L235-4) were less effective in controlling the tuber moth, but were significantly different from the non-transgenic cultivars.

Agronomic evaluation of the Bt-transgenic potato lines was initiated in Michigan in 1994. Yearly agronomic evaluations have been conducted at this location and the trial size has increased to accommodate the number of Bt-lines being tested. These trials have shown that many of the Bt transgenic lines perform similar to their non-transgenic cultivar. These trials also served as a training site for the AGERI scientists for biosafety and potato varietal assessment. With agronomic evaluations established in Michigan, seed tubers were produced for Egyptian field testing.

The first field test of genetically engineered potatoes in Egypt occurred in January 1997 at AGERI after the Egyptian biosafety regulations were established. The purpose of this trial was to evaluate an array of Bt-transgenic potato lines for field resistance to potato tuber moth. Fourteen lines were evaluated for foliar and tuber damage. To apply greater tuber moth pressure, the field was artificially inoculated during the season. Foliar mining was as high as 38 mines per 10 untreated plants, whereas the Bt-lines had as few as 1 mine per 10 plants. Non-transgenic tuber infestation was 80-92% (severe level of infection). In contrast, some of the Bt-transgenic lines had as little as 38% infection of the tubers. These results were very promising and expanded field trials were established for 1998 in Egypt. In February, 1999 AGERI trial was repeated and an insect and an agronomic trial were planted at the CIP Potato Research Station (located in the delta potato-producing region).

## Specific Objectives

- Continue genetic engineering development with emphasis on genes available for commercial development.
- Continue transformations with new gene/vectors for varieties important to Egypt.
- Examine the individual foliar expression levels of new transformations in lab and field tests.
- Evaluate the effectiveness of individual and combined resistance factors under laboratory experiments.
- Develop linkages with US companies and Egyptian seed companies to promote the commercialization of desired potato lines.

- Evaluate different management strategies for maintenance of resistant potato varieties and their integration into IPM systems.

## Research Progress

A 1999 field trial was conducted in Egypt at AGERI and CIP-Egypt. The results show excellent control of PTM from several lines (Fig. 1and 2). Storage experiments were conducted with the harvested potatoes and we are waiting for the results of these tests.

Seed production increases for year 2000 field trials in Egypt were made at the MSU Potato Research Center, Montcalm Co., MI. Additionally, greenhouse tubers were produce by the private sector for field trials. We have 2000 seed pieces of Various 'Spunta' lines (SPG2, SPG3, SPS1, SPS4 and SP6a-3 [PVYcp/Cry5]). Our plans are to test these lines in Egypt for seed increase and test on commercial farms in year 2001. Planting these lines on commercial farms will allow growers to see the benefits of these lines and hasten commercialization.

Agronomic trials were conducted in Michigan testing the Cry5-Bt Atlantic lines. All lines were comparable to the non-transformed control. New constructs using four different for BtCry5 expression vectors were developed. The different promoters include CAMV35s, Gelvin super promoter (GSP), Patatin, and Ubiquintin3. The Ubiquitin3 promoter was developed by USDA and thus eliminates several IPR restraints. This promoter is being used to design a freedom-to-operate vector, which may hasten commercialization of our transformed potato lines. We are currently conducting detached-leaf feeding bioassays in the lab with 'Spunta' lines transformed with the different constructs.

New constructs using the same four promoters have been developed for codon-modified Cry1Ac (J. Kemp, NMSU) expression. Potato transformation will begin in the near future.

'Atlantic' and 'Lady Rosetta' transformations with a Cry5 Gus minus vector have been completed. These lines are important chip varieties in Egypt and are key for Egyptian commercialization. Additionally, MSG274-3 (MSU late blight resistant line) has been transformed with the Cry5 Gus minus construct.

Laboratory PTM feeding bioassays will begin in the Spring of 2000 and seed increase is planned for the Michigan 2000 growing season. Year 2001 field trial in Egypt is planned.

Transformed tissue culture plantlets of 'Spunta' and 'Atlantc expressing Cry5 were sent to The International Potato Center (CIP), Lima, Peru for testing their populations of PTM. These tests are almost complete and results will be reported.

## Discussion/Implications

Potato transformation with our different promoters and new genes are showing excellent control of PTM. Seed increases of these lines will allow for test on grower's farms in Egypt. This is a important step to commercialization as it allows the producer the opportunity to observe first hand the benefits of the product. Transforming 'Atlantic' and 'Lady Rosette' is also an important step towards commercialization in Egypt as these varieties are very important in the Egyptian chip industry.

## Highlights

As stated above and additionally, the previous field and future field tests conducted in Egypt. D. Douches has had discussions with Monsanto® regarding their interest in commercialization.

## Publications

Ahmed Mohammed, D. S. Douches, W. Pett, E. Grafius, J. Coombs, Liswidowati, W. Li, and M. A. Madkour. 1999. Evaluation of Potato Tuber Moth Resistance in Tubers of *Bt-cry5* Transgenic Potato Lines. Accepted by J. Econ. Ent.

Grafius, E.J. et al.. 1999. Genetic engineering of potato for resistance to potato tuber moth: Egypt field trial results. Presentation at Entomological Society of America Meetings Atlanta, GA.

Douches D. Et al .. 1999. Presentation of the Egypt Project at PAA and NCR-84.

Pett, w. et al. 1999. Egypt field trial results presented at Manejo integrado de plagas de los principales cultivos Andinos. Cusco, Peru

## Travel

W. Pett to AGERI May, 1999
D. Douches to AGERI June, 1999
T. El-Nasr to MSU in July, 1999

Figure 1. 1999 CIP PTM TRIAL

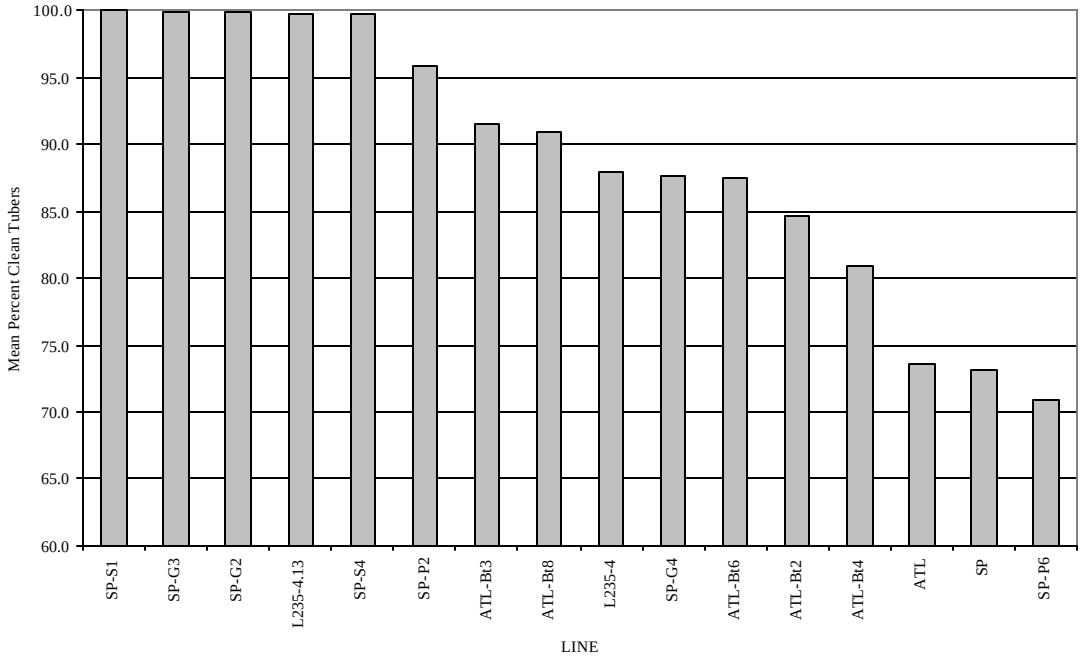
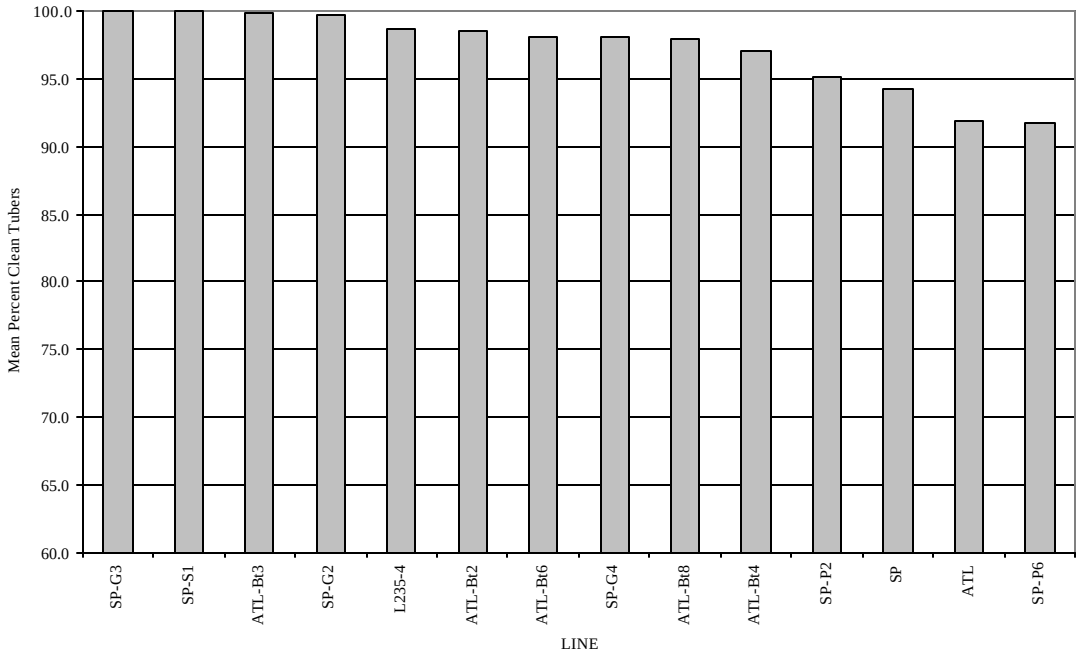



Figure 2. 1999 AGERI PTM TRIAL



# Managing Natural and Engineered Resistance in Potato to Potato Tuber Moth


## Egyptian Team

Magdy Madkour, AGERI
Taymour Nasr El-Din, AGERI
Emad Anis, AGERI

## US Partners

Dave Douches
Edward Grafius
Walter Pett


## Background

Potato (*Solanum tuberosum* L.) is an important vegetable crop in Egypt. The area of potato under production has reached 292,000 hectare/year over three seasons ( i.e. winter, spring, and summer). The total production is around 2.5 million tons annually with the winter season crop used mainly for export. Egypt exports 250,000 tons to Europe and the Arab countries. The yield is affected by infestation with potato tuber moth (PTM) *Phthorimaea operculella* (Zeller).The insect attacks potato plants in two ways: i) by mining the foliage and ii) by feeding on tubers. Therefore, it is an important pest both in field and storage.

## Objectives

1. Develop and standardize bioassay procedure for testing Bt varieties against PTM and other insect pests.
2. Improve regeneration and transformation system for Egyptian potato cultivars.
3. Evaluate Bt-transgenic lines developed by the project in the field.

# Research Achievements

## Transformation of potato ( *Solanum tuberosum* L.*)*:

### *Agrobacterium tumefaciens* culture

A single colony from the *Agrobacterium* strain containing plasmid with *Bt* genes were grown in 2 ml of LB liquid medium containing 50 µg/ml kanamycin. The *Agrobacterium* cultures were grown over night at 28 °C in an incubator shaker at 200 rpm. Next day 300 µl from over night bacterial cells were transferred to 30 ml of LB liquid medium and incubated at 28 °C for 4 hours at 200 rpm. until (O.D) reached 0.5-1.0 at wavelength of 600 nm. Five constructs received from MSU were used in these experiments (pBIML5, pBIML2, pBIML1, pBIML4, pBICry v).

### Transformation of potato leaves

In vitro leaves of plantlets (5-6 mm in diameter) were selected and collected in sterile Petri dishes under aseptic conditions. The upper and lower parts of the leaflets were cut out with sharp blade then the leaves were transferred into petri plate containing 30 ml of an over night *Agrobacterium tumefaciens* culture, and incubated approximately for 10 min. After incubation, the excess bacteria was blotted on sterile filter paper and the leaves were spread out onto the regeneration media and incubated for 3 days at 25 ± 1 °C in the dark. After that, leaves were rinsed in sterile distilled water and blotted dry on sterile filter paper in the laminarflow hood. Five leaves were planted on callus medium containing MS salts and 100 mg/l kanamycin monosulfate and 200 mg/l cefotaxime sodium salt. The antibiotics were added through disposable filter (0.22 µm) to the autoclaved medium under sterile conditions, and incubated at 25 ± 1 °C in the dark. After 10 days, leaves were transferred into regeneration medium containing 100 mg/l kanamycin monosulfate and 200 mg/l cefotaxime sodium salt. The cultures were kept in the growth chamber at 25 ± 1 °C which is illuminated with fluorescent tubes for 16 hours per day at 3000 Lux. After 72 days, the small shoots were taken and placed on propagation medium and incubated for 6-7 weeks.

Results of regeneration and transformation experiments for Spunta and Desiree cultivars are tabulated below (Fig, 1).

## Transformation of potato leaves

| Construct | Spunta c v. | | Desiree c v. | |
|---|---|---|---|---|
| | Callus | Shoots | Callus | Shoots |
| **Cry V** | 7 | 4 | 8 | 9 |
| **pBI ML5** | 8 | 13 | 10 | 27 |
| **pBI ML1** | 4 | - | 5 | - |
| **pBI ML2** | 2 | - | 3 | - |
| **pBI ML4** | 2 | - | 3 | - |

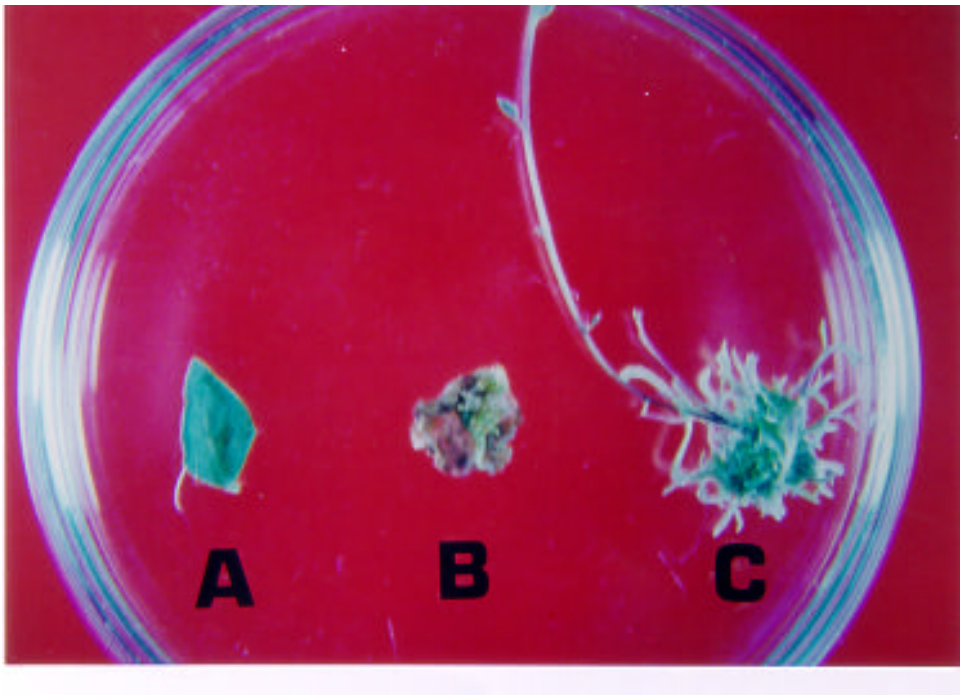



**Fig (1): *In vitro* regeneration steps in tetraploid potato plants, cultivar Desiree.**

(A): Establishments of leaflets 25 day-old on callus initiation medium.

(B): Callus initiation observed on the edges tissue.

(C): Shoots proliferation and development on regeneration medium.

Results revealed that the technology for regeneration and transformation of potato are well established at AGERI laboratory. Methods of regeneration using *in vitro* leaflets and *Agrobacterium transformation* system were utilized successfully on cultivars Spunta and Desiree are commonly used in Egyptian agriculture. Other important cultivars will be covered in the future work plan.

## Field trials

Since January 1997, the ABSP project provided potato transgenic lines to test their resistance to PTM in field trials in Egypt after obtaining clearance from USAID and the Egyptian National Biosafety Committee (NBC). Three tests were carried out at AGERI Experimental Plots (i.e. 1997, 1998, and 1999) whereas two trials were conducted at CIP station in the middle of Delta for evaluation of the level of resistance to potato tuber moth (PTM).

Field trials in 1999 involved transgenic lines with different Bt -constructs and various gene promoters. The lines were derived from Atlantic a processing cultivar and Spunta a tuber stock cultivar. Field studies for testing transgenic and non-transgenic lines were conducted in February 1999. Potato tubers were planted in a randomized complete block design with 4 replications. Treatments included transgenic with various modified gene and non-modified constructs. The insect damage on foliage was evaluated 3 times during the growing season. The trials were harvested on June, 1999 and tubers were examined for PTM damage (Fig 2 - 4). Results of tuber infestation at AGERI and CIP are presented in Table (1). Results showed that Spunta lines viz. Sp G3, Sp S1 were the most promising lines and the Atlantic lines were significantly different compared to control.

Meanwhile, the performance of transgenic lines i.e., agronomic characteristics, were evaluated under field conditions in a separate experiment at CIP site. Observations were recorded during the growing season. In general there were differences between transgenic lines and non-transgenic control lines in terms of shape, size and color of harvested tubers.

Fig (2): Field trial at AGERI to evaluate Bt-resistance potato lines under Egyptian field conditions.

Fig (3): Inspection of harvested tubers for PTM damage.

**A**

**B**

Fig(4): Harvest of potato experiments at Kafr El-Zyat (CIP-station) and the field day in June 7, 1999 (A and B).

Table (1): Percentage of clean tubers in field trials harvested in June 1999.

| Lines | AGERI-PTM | CIP-PTM |
|---|---|---|
| Spunta control | 94.2 | 73.2 |
| Sp G3 | 100 | 99.9 |
| Sp-S1 | 100 | 100 |
| Sp-G2 | 99.7 | 99.8 |
| Sp G4 | 98.2 | 87.6 |
| Sp-P2 | 95.1 | 95.9 |
| Sp-P6 | 91.8 | 70.8 |
| Atlantic control | 92.0 | 73.6 |
| Atl-Bt3 | 99.9 | 91.3 |
| Atl-Bt2 | 98.5 | 84.7 |
| Atl-Bt6 | 98.2 | 87.7 |
| Atl-Bt8 | 98 | 90.9 |
| Atl-Bt4 | 97 | 80.9 |
| L235-4 | 98.7 | 97.9 |
| L235-4.13 | - | 97.8 |

Some healthy tubers presenting the transgenic and non-transgenic lines were stored in a traditional storage facility (Nawalla), which has no cooling system. Tuber damage was examined during the storage period. Table( 2 ) represents the results of inspection. The results revealed that the control lines i.e. Atlantic, Spunta, and Diamant are more susceptible to infestation compared to transgenic lines.

Table(2): Results of storage experiment on transgenic and non-transgenic potato lines.

| | | 1st inspection 7/7/99 | | | 2nd inspection 28/7/99 | | | 3rd inspection 28/7/99 | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Varieties | Total | Non-Infected | Infected tubers | Rotted tubers | Non-Infected | Infected tubers | Rotted tubers | Non-Infected | Infected tubers | Rotted tubers |
| Atl | 42 | 7 | 30 | 5 | 3 | 4 | - | - | 3 | - |
| Atl. BT2 | 51 | 25 | 25 | 1 | 7 | 18 | - | 6 | 1 | - |
| Atl-Bt3 | 51 | 30 | 20 | 1 | 14 | 15 | 1 | 6 | 6 | 2 |
| Atl Bt4 | 38 | 8 | 25 | 5 | 8 | - | - | 7 | 1 | - |
| Atl Bt6 | 86 | 23 | 56 | 7 | 18 | 5 | - | 10 | 8 | - |
| Atl Bt8 | 52 | 28 | 21 | 3 | 17 | 10 | 1 | 5 | 11 | 1 |
| L235-5 | 119 | 19 | 80 | 20 | 9 | 10 | - | 7 | 2 | - |
| Diamant | 110 | - | 92 | 18 | - | - | - | - | - | - |
| Spunt | 110 | 20 | 80 | 10 | 13 | 4 | 3 | 6 | 7 | - |
| Sp G2 | 150 | 141 | 6 | 3 | 140 | 1 | - | 135 | 4 | 1 |
| Sp G3 | 203 | 181 | 20 | 2 | 180 | - | 1 | 168 | 12 | - |
| Sp G4 | 133 | 131 | 1 | 1 | 126 | 3 | 2 | 120 | 4 | 2 |
| L235-4-13 | 235 | 220 | 5 | 15 | 118 | 2 | 5 | 198 | 3 | 7 |
| Diamant | 132 | - | 120 | 12 | - | - | - | - | - | - |
| Spunta | 47 | -43 | 4 | - | - | - | - | - | - | |
| Sp S1 | 152 | 148 | 2 | 2 | 147 | 1 | 5 | 130 | 9 | 3 |
| Sp S4 | 124 | 121 | 1 | 2 | 119 | 1 | 1 | 101 | 15 | 3 |
| Sp P2 | 228 | 11 | 215 | 2 | 4 | 7 | - | - | 4 | - |
| L235-4-13 | 235 | 220 | 10 | 15 | 199 | 10 | 1 | 190 | 4 | 5 |

## Travel and Visits

Dr. T. Nasr El-Din and Mr. H. El-Shishtawy visited field sites at MSU during their participation in the International Short Course in Food Safety (July, 11-16,1999).

## Future plan of work

***In the Laboratory*:**

1. Apply the transformation technique on wide a range of potato cultivars grown in Egypt such as Cara, Draga, Nicola and Alpha.
2. Use of Bt modified constructs obtained from MSU counterpart and other sources in transformation of potato.
3. Evaluation of transgenic potato developed from the program for Bt-resistance using molecular analysis.

4. Start to produce transgenic potato lines resistant to both Bt and virus diseases.

***Field trials*:**

1. Conduct small and large-scale trials for transgenic potato cultivars under different field conditions in Egypt.
2. Evaluate new transgenic potato lines for their level of insect resistance and agronomic characteristics.
3. Start to commercialize the transgenic potato lines in Egypt with the help of office of the technology transfer at AGERI.

***Future Training and visits*:**

1. One postdoctoral degree from AGERI to MSU for 6-8 months in the field of construct designing and transformation techniques.
2. Visits to field trials at MSU and other locations for short periods (1-2 weeks).

# Molecular Characterization of Insect Midgut Toxin Receptors for Circumventing Resistance to Toxins of *Bacillus thuringiensis*


## Principal Investigator

Lee A. Bulla, Jr., University of Texas at Dallas


## Background and significance

There is increasing concern by scientists, agriculturists and environmentalists about the potential of insects developing resistance to *Bacillus thuringiensis* (Bt) because of its widespread use as an insecticide and in transgenic plants. Bt has been the basis of a variety of biopesticide formulations that have been produced commercially during the past 20-30 years. These biopesticides have been used extensively in the United States and in a number of other countries throughout the world. Transgenic plants carrying the toxin genes of Bt have been introduced into the United States and efforts are underway to utilize such plants in Egypt and the Middle East. Several Bt biopesticides have been marketed and used in Egypt and the Middle East for crop protection. One insect, the cotton leafworm (*Spodoptera littoralis*) which is a major problem in horticultural crops such as tomatoes, potatoes, and cucurbits as well as in corn, is effectively controlled by Bt insecticidal toxins. Recently, however, the cotton leafworm has exhibited some resistance to Bt toxins. Therefore, it is important to gain a better understanding of the molecular properties of the receptors that bind Bt toxins and that mediate toxicity to insects such as the cotton leafworm.

Several Bt toxin-binding proteins have been identified in various insects. However, only one receptor molecule, BT-$R_1$ from the tobacco hornworm (*Manduca sexta*), has been cloned, sequenced and determined to mediate insect toxicity. Several homologues of BT-$R_1$ also have been identified by the principal investigators in insect pests important to Egyptian and American agriculture. Because resistance to Bt insecticides is a distinct possibility in these insects, it is imperative to gain a better understanding of the nature and basis of such resistance. The toxin-binding site of BT-$R_1$ has been determined by collaborative efforts between the co-principal investigators, and, its physical structure will be determined in the near future. The immediate next steps are to purify, clone and characterize the BT-$R_1$ homologue that also has been identified in the cotton leafworm by the principal investigators. Knowing the mechanism of binding will allow us to ascertain the parameters that dictate normal binding versus abnormal or reduced binding in resistant insect species. Once understood, new insecticidal Bt toxins can be either synthesized artificially, genetically engineered, or purified from strains and isolates of Bt that are effective against resistant insects. Thus, resistance to Bt toxins by insects can be circumvented and insect control can be continued in an environmentally friendly manner.

## Goals & Objectives

- Select and rear resistant/tolerant colonies of the cotton leafworm
- Clone and express those genes that encode BT-$R_1$ homologues in the cotton leafworm
- Determine the primary nucleotide and amino acid structures of the BT-$R_1$ homologues purified from susceptible and resistant strains of the cotton leafworm
- Identify and sequence the binding sites of the BT-$R_1$ homologues
- Calculate the binding dissociation constants for the corresponding Bt toxins
- Construct synthetic peptides based on the primary sequences of the susceptible and resistant BT-$R_1$ homologues
- Bioassay the peptides for insect toxicity
- Select the most toxic peptides for further studies and testing

## Results of previous research

- A single binding protein (210 kDa) was purified from the lepidopteran insect, M. sexta, that is specific for the Cry1Aa, b, and c toxins of Bt. The protein is sensitive to proteolytic digestion and is rich in acidic amino acids. The receptor, called BT-R1, has a pI of ~5.5 and is glycosylated. Radiolabeled toxin binds to the protein with a Kd value of 708 pM and can be specifically blocked by unlabeled Cry1A toxin but not by toxins from other subspecies of Bt that are effective against coleopteran and dipteran insects.
- A cDNA that encodes BT-R1 was cloned and expressed in heterologous mammalian cell systems. The 210-kDa receptor is a membrane glycoprotein that specifically binds the Cry1A toxins of Bt and mediates insect death. BT-R1 shares sequence similarity with the cadherin superfamily of proteins.
- Characterization of the expression of BT-R1 for the Cry1Ab toxin of Bt revealed that the receptor is highly regulated. Developing tobacco hornworm larvae exhibited a significant increase in their level of BT-R1 mRNA and protein expression as they mature. Furthermore, BT-R1 expression is highly localized to the midgut of M. sexta larvae, which has been shown to be the site of toxin activity. BT-R1 expression in the midgut increases seven-fold from first through fifth instar with a concomitant decrease (46-fold) in the susceptibility of fifth instar larvae to the Cry1Ab toxin of Bt. Based on these observations, the molecular mode of action of the Cry1Ab toxin, most likely, is mediated through the disruption of the normal physiological function of BT-R1 and/or additional proteins associated with it. To harm the insect, the toxin must be present in the midgut of M. sexta larvae at levels sufficiently high enough to saturate the receptor population and impair normal midgut cell function which is critical to the health and normal development of the hornworm.
- When BT-R1 is expressed in artificially cultured insect cells (Sf21), a significant amount of the protein is produced as a soluble protein in the culture medium. The soluble fraction of BT-R1 accounts for ~60 percent of the total expressed protein. Soluble BT-R1 is stable in the culture medium for at least three days at 25oC and for up to seven days at 4oC. This stability will facilitate the accumulation of the protein over an extended period and render it suitable for further molecular characterization. In other words, the expression system in Sf21 cells provides an excellent means for large-scale production and purification of BT-R1 and its homologues from the

tobacco hornworm and the cotton leafworm, respectively. Having large amounts of these receptor molecules will expedite their further characterization.

## Work in progress

- Establish colonies of the cotton leafworm and tobacco hornworm that are resistant to or tolerant of Cry1A toxins - AGERI
- Perform ligand blot analyses of brush border membrane vesicles (BBMV) prepared from both insects to determine the presence of BT-R1 homologues - AGERI
- Perform competition inhibition assays with unlabeled Cry1A toxins using frozen BBMV preparations from the cotton leafworm obtained by AGERI – UT

## Research results

Due to the late start of the project in 1999, there is no progress to report at this time. However, the necessary personnel have been hired and the above areas of work have been initiated and are underway.

# Molecular Characterization of Insect Midgut Toxin Receptor for Circumventing resistance to *Bacillus thuringiensis*

**Dr Yehia A. Osman, AGERI**

*Bacillus thuringiensis* (Bt) is a natural entomopathogen and is known to produce insecticidal proteins (ICP) during the sporulation stage of growth. These ICPs do kill insect larvae belonging to insect orders: Lepdiptera, Diptera and Coleoptera. This would make Bt a very useful tool in biological control of agricultural pests and a major component of an integrated pest management program (IPM). The main objective of this research project is the establishment of a resistant and/or tolerant cotton leafworm (*Spodoptera littoralis*) colony to the insecticidal crystal protein (ICP) of *Bacillus thuringiensis*. This would enables us to study the mechanism of tolerance and/or resistance to the ICP such as the Cry1 toxin family.

We, at AGERI, established a system to raise tolerant and/or resistant *S. littoralis,* using the ICP belonging to the Cry1 family such as Cry1C, Cry1Ab, and Cry1Ac. We started the colony by using very low concentrations (1, 2, 5, and 10 ppm) of the toxins incorporated into the artificial diet. The survived larvae are transferred to fresh medium containing the toxin(s) for five generations before we transfer to a diet containing higher concentrations. The colony, after initial difficulty, is proceeding well. This will help us ultimately understand the mechanism by which an economically important insect will become resistant and/or tolerant to Bt toxins. The studies will proceed according to the prescribed protocols stated in the initial proposal.

# Tomato transformation for Development of Geminivirus Resistance


## Principle Investigator

Naglaa A. Abdallah, AGERI


## Overall Project Objectives

Whitefly-transmitted geminiviruses (WTGs) are a major threat to the productivity and quality of tomato grown in subtropical and tropical regions. Economic loss due to the geminivirus TYLCV infection and whitefly infestations in tomato plants often range between 60-100% in Egypt. Outbreak of the whitefly vector was recently recorded due to the development of resistance to insecticides, causing the virus to spread rapidly. To date, there are no reliable means for controlling or reducing viral infectivity and traditional breeding methods have failed in producing geminiviral resistant crops. Molecular genetics and plant transformation techniques provided new tools to introduce foreign genes into plant tissues without comprising other economic characters of the existing cultivar. The suicide cell strategy was used to develop genetically modified tomato plants resistant to TYLCV.

## Research Progress

The viral transcriptional activator promoter (CP-promoter) was cloned upstream of the cytotoxic RNase barnase gene to insure expression of the transgene into the virus infected cells. Since the 5'end of the promoter is not identified, two constructs (Fig. 1) were performed, one with the full C4-ORF (pTYSUC) and the other with 3'C4-ORF truncated end (pTYSUC$\Delta$70). The two constructs were introduced into the local tomato cultivar GH75 using *Agrobacterium*-mediated transformation.

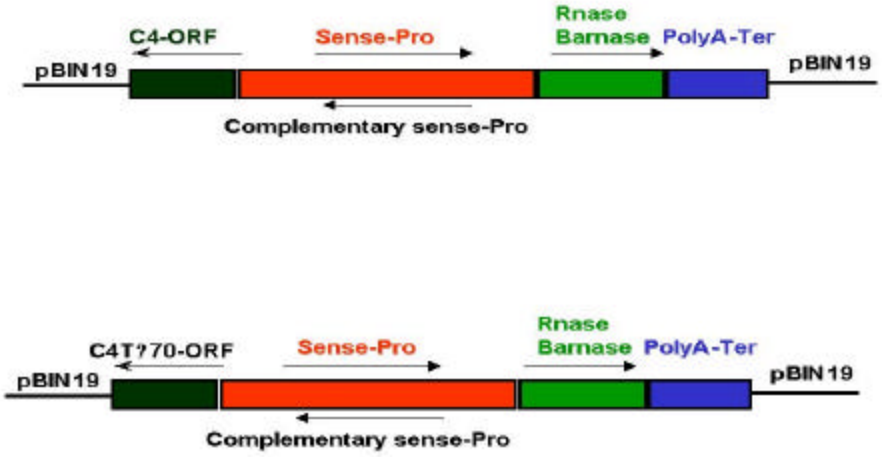
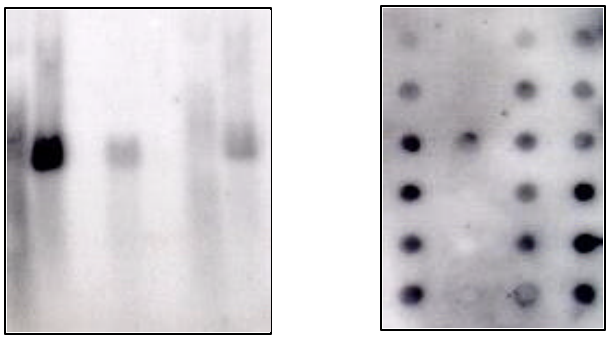


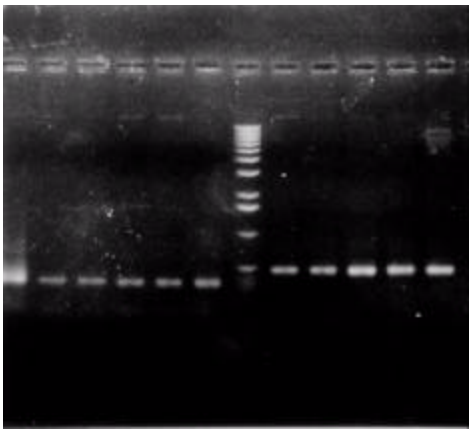

Figure (1) : Genetic map of the constructed expression cassette carrying the barnase gene under the control TYLCV sense-promoter and the sense-promoter and the C4TΔ70 gene under the control of the complementary sense-promoter.

### A. Evaluation of the transgenic plants at the $R_0$ stage

Transformed plants were kept in greenhouse biocontainment and evaluated for the presence of the introduced gene using PCR,DNA hybridization (Fig. 2) and Western blot analysis (Fig. 3). Viruliferous whiteflies were used to inoculate the plants and symptoms appearance were scored weekly. No symptomes were observed on the transgenic tomato carrying the suicide cell constructs (pTYSUC & pTYSUCΔ70) at this level and seeds were collected from those plants (Fig. 4)



(a)



(b)

Figure (2): Evaluation of the $R_0$ tomato plants using: (a) DNA hybridization for DNA extracted from transformed plant to detect the introduced gene using Southern and dot blot hybridization. (b) PCR for DNA extracted from the transformed plants using specific primers.

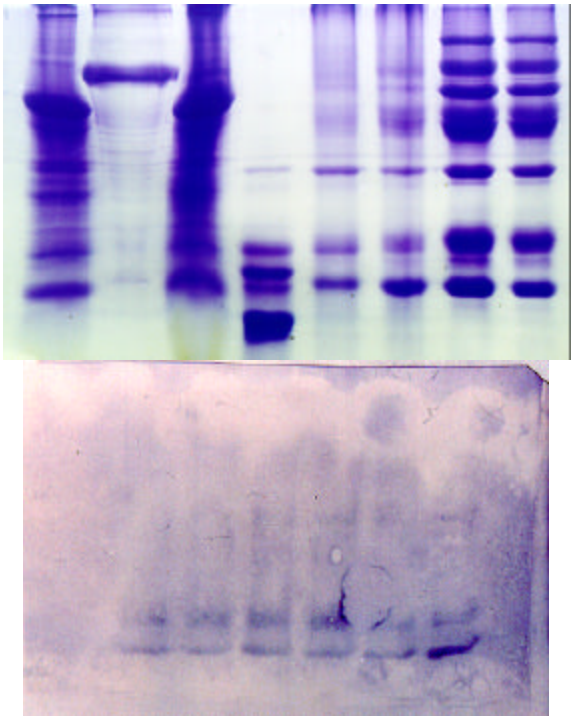


Figure (3): SDS-PAGE for total protein extracted from transformed tomato plants (a) and it Western blot using C4 antiserum (b).

Figure (4): Transformed tomato plant with the suicide cell strategy after inoculation with the viruliferous whiteflies showing no symptoms.

**B. Evaluation of the transgenic plants at the $R_1$ stage**

Seeds collected from the $R_0$ plants were cultivated in greenhouse biocontainment and evaluated for the presence of the introduced gene using PCR analysis (Fig. 5). Viruliferous whiteflies were used to inoculate the plants and symptoms appearance were scored weekly (Fig. 6). Segregation of the character was observed and calculated to be 1:1 indicating that the transgenic plants have one copy of the introduced gene. Seeds from the viral resistant plants which show no TYLCV symptoms will be cultivated to obtaining homologous plant for the introduced construct.

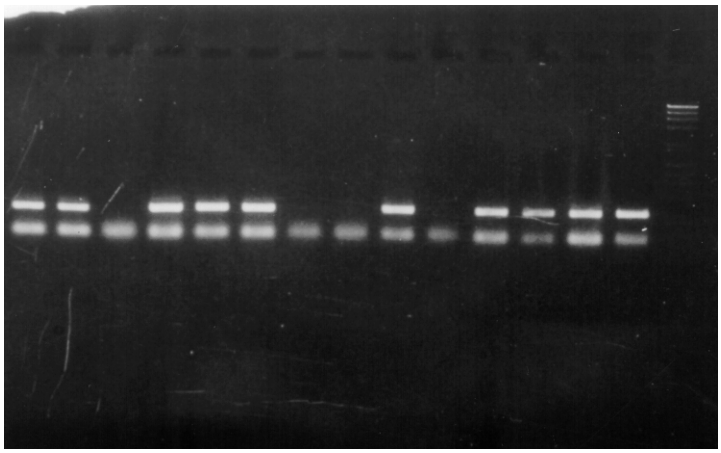


Figure (5): PCR for DNA extracted from the $R_1$ transformed plants using specific primers.

Figure (6): Transformed $R_1$ tomato plants using the suicide cell strategy showing no symptoms after 8 weeks of inoculation with viruliferous whiteflies.

**C. Transformation of the CastleRock cultivar with the suicide cell strategy**

The CastleRock cultivar is commonly used in the open field cultivation in Egypt therefore, it was selected to be transformed with the suicide cell construct to gain resistance against TYLCV. *Agrobacterium* carrying the plasmid pTYSUCΔ70 was used to transform the CastleRock and seeds obtained from the symptomless transformed plants will be collected and evaluated for the stability of the introduced resistance in the following generations.

# Developing Drought And Salinity Tolerant Wheat And Tomato For Egyptian Agriculture


## Egyptian Principal Investigators (AGERI)

Prof. Dr. Magdy Madkour

Dr. Ahmed Bahieldin

Dr. Ashraf Haider

## US Principal Investigator

Prof. Desh Pal S. Verma, Ohio State University


## Overall Project Goals

The ultimate goal of this project is to enhance osmotic stress tolerance in Egyptian wheat and tomato crops. This will be achieved by over expressing the key regulatory enzymes of the proline biosynthesis and sulfer assimilation pathways. We will directly determine whether elevated levels of proline and active sulfer confer drought and salinity tolerance in two plant systems, i.e., wheat and tomato. Attempts will be made to find gene(s) able to convert proline into proline betaine.

## Justification For The Proposed Project

This project is based on the idea that accumulation of proline, a known osmolite, can enhance drought and salinity tolerance in crop plants and extend the survivability of crop plants under stress conditions. During the last 5 years, Professor Verma's group (our collaborator) has not only isolated all genes involved in proline biosynthesis, but also demonstrated that P5CS is a rate-limiting enzyme in proline synthesis and over expression of this enzyme produces more proline if sufficient nitrogen is available. They have identified a bifunctional enzyme P5CS able to make P5C from glutamate and which limits proline synthesis. Furthermore, they have removed feedback control of this enzyme by proline. This mutagenized enzyme is active up to almost 1M concentration of proline. Recent unpublished data suggest that overexpression of mutaginized P5CS can produce more proline in plants. They have obtained a patent on this gene (Patent# 5,344,923; Sept 6, 1994). This gene has been licensed to an Australian company for introducing in forest crops.

A rice *HAL2*-like (*RHL*) cDNA (Peng and Verma, 1995) has been isolated and characterized by Professor Verma's group, this gene supports the growth of cells under high salinity stress. They have further shown that availability of active sulfur was essential to overcome oxidative stress imposed by salinity and drought stresses. Over expression of this gene in plants results in accumulation of glutathione which also reduces oxidative as well as salt stress.

## Background

Over 50 million hectares of agricultural land throughout the world suffer from excessive salt accumulation resulting in poor crop productivity. An equally large area of land mass is affected by recurrent drought. Drought and salinity are formidable problems to the development of new varieties that can give sufficient yield under water stress conditions. Undoubtedly, these two factors are the major obstacles in improving crops in the Middle East and Africa and offer a challenge to genetics and biotechnology.

Recent studies in Professor Verma's laboratory, our collaborator at the Ohio State University, have demonstrated that by overexpressing the gene encoding Δ1-pyrroline-5-carboxylate synthetase (*P5CS*), the first enzyme of the proline biosynthesis pathway, one can enhance proline production in transgenic plants. His group has further demonstrated that if the feedback inhibition of *P5CS* by proline is removed, the level of proline can be further increased 2 to 3 folds of that produced by the wild type enzyme. This was achieved by first determining the feedback inhibition domain of *P5CS* followed by site directed mutagenesis and overexpressing the mutated gene. They are in the process of converting proline to proline betaine, which is over 100 times more effective in osmoprotection than proline. If a part of the proline pool could be converted to proline betaine, it may provide us with an "*ultimate osmolyte*" easy to be synthesized from naturally accumulated proline. They have demonstrated that the excess of proline is rapidly oxidized once stress conditions are removed, and therefore the biosynthesis of proline and proline betaine appears a most attractive route for developing crops able to withstand higher osmotic stress environments.

The latest advances in wheat transformation technology (Vasil *et al.,* 1993; Weeks *et al.,* 1993) and molecular and physiological studies of the genes involved in osmotic responses to stress conditions will allow us to introduce genes to improve water-stress tolerance in Egyptian and American cultivars. Transformation experiments using these genes in dicotyledonous plants have shown that increased stress tolerance can be achieved. Promising data on introduction of this gene in rice also looks very promising (Verma *et al*, unpublished data). Results of these experiments also have direct potential for development of salt- and drought- tolerant wheat cultivars for improving wheat productivity in Egypt.

Collaboration between AGERI and Ohio State University started by transforming immature embryos of Egyptian and American bread wheats with genes for salt and drought tolerance. At AGERI, many putative transgenic plants of both cultivars have been obtained. In addition, we have been successful in increasing regeneration and transformation efficiencies for both cultivars by shortening the selection period, bombarding young immature embryos, using low dicamba concentrations (Bahieldin *et al.*, in preparation) and allowing callus to recover for a week after bombardment. All necessary tools to accomplish the objectives of this project are available at AGERI and Ohio State University improving the chances of success of this project.

## Rationale

Drought and salinity are formidable obstacles for breeding crops that can withstand these stresses which are major obstacles in crop productivity under water-limited environments such as that exists in much of the Egyptian Agricultural land. To survive under water stress, organisms have evolved many strategies that involve accumulation of specific osmotic solutes or osmolytes. Among the energy-rich metabolites proline and betaines are most prevalent (Yancey *et al*., 1982; Goas *et al*., 1982; McCue and Hanson, 1990;

see Delauney and Verma, 1993). A marked increase (up to 100-fold) in free proline concentration occurs in many mesophytic flowering plants during severe water deficit (Hanson and Hitz, 1982; Delauney and Verma, 1993). Proline also accumulates in rice and maize under osmotic stress conditions (Ober and Sharp, 1994, Chou *et al*., 1990). Tomato cells cultured in a low water potential environment show rapid accumulation (up to a 300-fold) of proline (Handa *et al*., 1986). Kinetic studies using $^{15}$N-labeling (Rhodes *et al*., 1986) have shown that the total increase in proline accumulation occurs by a ten-fold change in the rate of synthesis, with concomitant reduction in the rate of degradation.

## Previous Research

Field crops are often prevented from achieving their full genetic potential due to biotic or abiotic environmental stresses. Water stress (hyperosmotic) caused by drought and salinity is the most important abiotic factor limiting plant growth and crop productivity worldwide (Boyer, 1982). Arable land acreage is limited in Egypt due to the lack of water needed for irrigation. The amount of High Dam water available is only sufficient for the irrigation of additional two million feddans of cultivated land along the north coast of Egypt. In addition, agricultural development in North Sinai as well as Century Project of the New Valley in the western desert "Tushky" will depend mainly on irrigation with mixed fresh and drainage water, which raises the need for developing crop cultivars with increased salt and drought tolerance. The gap between future supply and demand in wheat and tomato (strategic commodities), makes it imperative to increase these areas where suboptimal conditions prevail, i.e., water deficit, salinity and high temperature.

In microorganisms and plants, proline is synthesized primarily from glutamate γ-semialdehyde and its spontaneous cyclization product Ä,1-pyrroline-5-carboxylate, P5C (Adams and Frank, 1980). Proline biosynthesis is regulated by feedback inhibition of γ-glutamyl kinase (γ-GK), the first enzyme of the pathway, encoded by the *proB* gene; a single mutation in this gene results in proline overproduction (Dandekar and Uratsu, 1988, Csonka *et al*., 1988), allowing cells that harbor the mutant gene to grow in medium containing up to 0.9 M NaCl. In higher organisms, another route for proline synthesis occurs from ornithine via P5C to proline. Arginine can be easily converted to ornithine by arginase. γ-GK catalyzes the ATP-dependent phosphorylation of L-glutamic acid and is inhibited by proline. The second enzyme, γ-glutamyl phosphate reductase encoded by the *proA* gene, functions as a complex with the γ-GK. P5C is reduced to proline by P5C-reductase encoded by *proC* gene. Complementation of a mutation in the *proC* gene of *E. coli* with soybean *P5CR* cDNA (see Delauney and Verma, 1990) has been achieved. Verma's group has isolated the genes encoding the first two enzymes of this pathway using the same approach (Hu *et al*., 1992). Using a trans-complementation strategy, they isolated ornithine aminotransferase (*OAT*) gene and demonstrated that under stress conditions P5CS path is favored over OAT (Delauney *et al*., 1993).

## Specific Objectives

- Improve selection, transformation and regeneration efficiencies for major Egyptian wheat and tomato cultivars
- Transform major Egyptian wheat and tomato cultivars with genes for salt and drought tolerance

- Conduct greenhouse and field performance trials of regenerated transgenic plants at AGERI
- To introduce mutagenized *P5CS* (devoid of feedback control) and *HAL2* cDNA under the control of appropriate promoters in wheat and tomato.
- To isolate and characterize enzymes and their cDNAs for converting proline to proline betaine.
- Incorporate transgenic plants with improved salt and drought tolerance into ongoing Egyptian breeding programs.
- Enhance collaborative relationships coexisting with the state-of-the-art technology and facility that will improve modern genetic technology in Egyptian agricultural research.

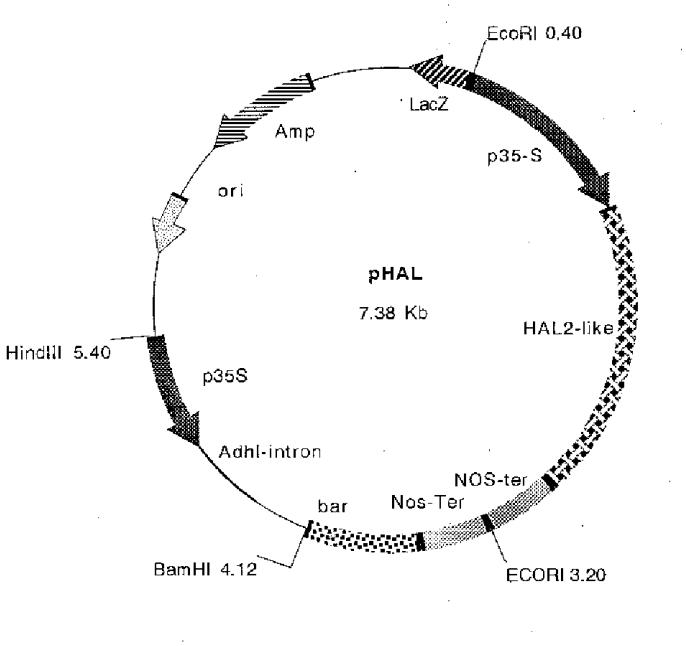
## Research Progress

### A. Gene construction:

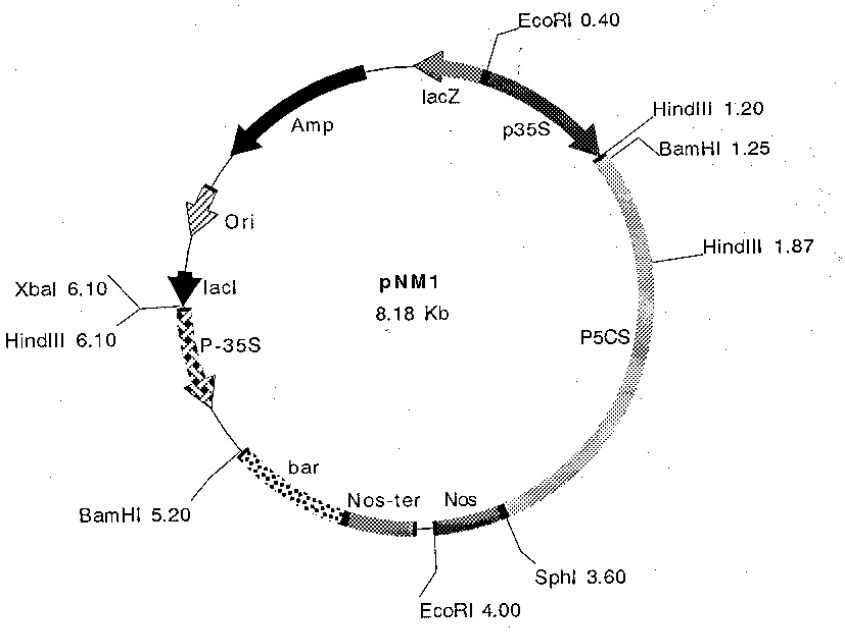
Two types of constructs were proposed be used for each gene. For protein production, cDNA sequences will be cloned into expression vectors in *E.coli*. The second set of constructs will be made for expression in plants.

**Transformation constructs:**

Two constructs bearing P5CS and HAL2 genes were finished. Figures 1 and 2 indicate the restriction maps of these two plasmids showing the promoters and gene cassette directions. A more detailed information regarding these two constructs will be provided in the next report.



Figures 1 and 2 restriction maps of the two plasmids, pHAL (7.38 kb) and pNM1(8.18 kb) showing the promoters and gene cassette directions of each.

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# Developing Drought And Salinity Tolerant Wheat And Tomato For Egyptian Agriculture

## Principal Investigators

**US**
Prof. Desh Pal S. Verma, Ohio State University

**Egypt**

Prof. Dr. Magdy Madkour
Dr. Ahmed Bahieldin
Dr. Ashraf Haider

## Project Goal

To enhance osmotic stress tolerance in Wheat and Tomato crops by over expressing proline biosynthesis genes and isolate other genes that can improve osmotolerance in crop plants.

## Importance of the problem and rationale of the approach

Water stress (hyperosmotic) caused by drought and salinity is the most important abiotic factor limiting plant growth and crop productivity worldwide (Boyer, 1982). Arable land acreage is limited in Egypt due to the lack of water needed for irrigation. The amount of High Dam water available is only sufficient for the irrigation of an additional two million feddans of cultivated land along the north coast of Egypt. In addition, agricultural development in North Sinai as well as the Century Project of the New Valley in the western desert "Tushky" will depend mainly on irrigation with mixed fresh and drainage water, which raises the need for developing crop cultivars with increased salt and drought tolerance. The gap between future supply and demand in wheat and tomato (strategic commodities) makes it imperative to increase cultivation in the areas where suboptimal conditions, such as water deficit, salinity, and high temperature, prevail.

## Previous Research

This project is based on the idea that accumulation of proline, a known osmolyte, can enhance drought and salinity tolerance in crop plants and extend the survivability of crop plants under stress conditions. We have isolated all of the genes involved in proline biosynthesis and demonstrated that P5CS is a rate-limiting enzyme in proline synthesis and that the overexpression of this enzyme produces more proline if sufficient nitrogen is available. We have identified a bifunctional enzyme P5CS able to make P5C from

glutamate and and shown that the activity of this enzyme limits proline synthesis. Furthermore, we have removed feedback control of this enzyme by proline . This mutagenized enzyme is active up to almost 1M concentration of proline. Recently submitted data suggest that overexpression of P5CS can produce more proline in plants (Hong et al. 2000) . We have obtained a patent on this gene (Patent# 5,344,923; Sept. 6, 1994).

In collaboration with AGERI we are proceeding to genetically engineer wheat and tomato plants with the ability to accumulate high levels of proline, which is expected to confer osmotolerance. This approach would have a major impact on crop productivity in Egypt. After novel genes are identified under this cooperative project, they will be transferred to AGERI for introduction into Egyptian wheat and tomato commercial cultivars. The methodology we will use for wheat and tomato can be considered as a model for other monocot and dicot crops. This technology will be transferred via the OSU technology transfer office to AGERI, which would further develop and commercialize it for Egyptian agriculture.

## Research Progress

Mr. Magdy Mahfouz from AGERI has been working in Dr. Verma's laboratory at the Ohio State University and has been engaged in the mutagenized P5CS gene construct for wheat and tomato transformation. This construct shall be tranferred to AGERI soon and will be introduced in wheat and tomato by our collaborator.

Many reports have stated that Citrus accumulate significant levels of osmolytes including mainly proline and proline betaine. As a part of this project we used a heterlogous functional complementation approach to fish out new genes that might be involved in conferring stress tolerance in crop plants. We used an E. Coli strain that is pro- and bet- due to the (gpt) lac deletion, which spans both of these loci to complement it with the Citrus cDNA expression library. Citrus cDNA expression library has been constructed . The cDNA library has been transformed into E. coli lacking bet operon strain using mass in vivo excision protocol. The threshold level of salt tolerance has been determined for this particular strain. We screened the library using this threshold level (0.45 M LiCl). We screened more than 500,000 clones. We fished out 13 clones which exhibit significant salt tolerance in E. coli. These clones will be soon sequenced. These sequences will be used to search homology against ESTs and protiens databases using BLAST system. Future work will involve using a number of genetical and biochemical approaches to assign a function to these novel genes.

## Implications of Results

preliminary data suggest that we are able to fishout new genes from Citrus that would be valuable for osmotolerance work and these genes once characterized may be considered for patent protection.

Highlights and Impact: The identification of novel genes for osmotolerance would have significant impact on the problem of drought and salinity resistance in crop plants and would be highly beneficial to the developing countries such as Egypt.

## Travel

None yet, plan to attend ASPP meeting in SanDiego in July 2000.

## Work Plan for Jan 2000- December 2001

We plan to characterize Citrus genes that have been identified so far and isolate more potentially useful clones by following oxidative stress strategy using a variety of agents that cause free radical production in plants. These genes will be characterized by using sequence homology search followed by assays for a potential enzymatic activity that may be revealed by sequence analysis. The functional assay will be performed by introducing these genes in Tobacco BY2 cells or transgenic plants. Patent application(s) will be filed with Ohio State Research Foundation, in case one or more of these genes turned out to be novel and effective in the bioassay.

Funds for this research activity were not released until December of 1999, but we had started the work with other resources and are now in a position to advance this project rapidly and are optimistic that significant results will be obtained under this collaborative project.

# Insect-Resistant Maize Subproject

## Principal Investigator

Dr. Terry Meyer, Pioneer Hi-Bred

## Project Partners

Dr. Yehia Osman, AGERI/Agricultural Research Center, Egypt
Dr. Magdy Madkour, AGERI/Agricultural Research Center, Egypt
Dr. Hanaiya El Itriby, AGERI/Agricultural Research Center, Egypt
Dr. Ebtissam Hussein, AGERI/Agricultural Research Center, Egypt
Dr. Lee A. Bulla, Jr., University of Texas, Dallas, Texas

## Overall Project Goal

Development of *Bt*-transgenic maize technologies for resistance to key insect pests

## Project Importance

Corn Borers (*Sesamia cretica, Ostrinia nubilalis, Chilo agamemnon)* are serious insect pests in much of the corn growing area of Egypt and are responsible for significant loss of yield. We propose to introduce into Egyptian commercial corn, (Pioneer proprietary germplasm already tested, registered and grown in Egypt) through available transformation technologies Bt gene(s) which are known to code for proteins that are lethal to lepidopteran species. These genes will be Pioneer proprietary Bt gene(s) and/or those Bt genes belonging to AGERI (i.e., novel Bts contributed by AGERI and discovered as part of this project). Transformed plants and their progeny will be tested for resistance to borer feeding and transgenic events conferring the most resistance to insect damage, and affording the best possible yield and agronomic traits will be identified and selected. Seeds will be registered and sold through Pioneer Hi-Bred through its normal distribution system in Egypt.

## Project Background

Maize growing areas in Egypt are infested with three major stem borer insect pests, all of which are lepidopterans. These are *Sesamia cretica, Ostrinia nubilalis* and *Chilo agamemnon,* with *S. cretica* causing the most damage. Damage due to *S. cretica* (pink borer) is incurred early in the plant life cycle and results can be catastrophic. The favored oviposition is the second and third leaves of a plant with extended height range of 20-100cm (Ismail, I., 1989). *S. cretica* infestation is greatest during the period from May to mid June when relatively low infestation levels can produce extensive damage. Larvae feed at the center of the developing plant causing "dead heart disease", which leads to plant death.

There are three generations of *Ostrinia nubilalis* in the Giza region of Egypt (Kira et al., 1975) where corn comes under attack from the early part of May until September (a five month period). In the South Delta region there are more generations, up to six (El-Sadeney, 1965), and the period of attack from *0. nubilalis* is extended. *C. agamemnon* has an economic threshold similar to that of *O. nubi1alis* i.e., approximately 20 egg masses/100 plants and eggs are laid at the 5-12 leaf stage. In the U.S., control of *O. nubilalis* has been routinely achieved by externally applied chemical insecticides and biological control agents including *Bacillus thuringiensis,* and in Egypt by the application of insecticides.

With the advent of plant biotechnology have come new tools to control damage from insect pests. Specifically, the introduction of Bt-transgenic maize and other crops during the past few years afforded farmers a means to produce good crops without the use of more traditional and relatively toxic organochemicals for insect pest management. However, these technologies are expensive to develop and have been undertaken in relatively few regions of the world, as yet. Furthermore, the development of transgenic agricultural products depends on sound intellectual property rights (IPR) and regulatory approval policies—the development of which has lagged in many parts of the world. Within the context of the ABSP/USAID program, it should be possible to foster the development of these key elements of agricultural biotechnology, especially in the countries supported by this program.

## Rational for Approach

Transgenic corn plants expressing an insecticidal protein derived from *Bacillus thuringiensis,* (Bt) have been field tested and shown to resist insect feeding (Koziel et al, 1993). The insect toxicity of Bt resides in large proteins which have no toxicity to beneficial insects, other animals or humans (Wilcox et al, 1986). Since corn borers feed on corn developmental stages ranging from seedling to maturity, resistance would be most effective if it extended throughout plant growth. The expression of transgenes can be controlled in the plant by putting them under the control of specific promoters (Benfey and Chua, 1989). With the appropriate promoters, expression of Bt genes can be sustained throughout the life of the plant.

Several routes exist to the transformation of corn (see Wilson et al, 1994 for review). The most widely used is particle bombardment of cell cultures or of immature zyogotic embryos (Gordon-Kamm et al, 1990; Koziel et al, 1993, respectively). There is some genotype restriction of lines which can be transformed in this manner. Transgenic maize plants can also be produced by tissue electroporation (D'Halluin et al, 1992). Here again, embryogenic callus is required. Protoplasts have also been used as the starting point for gene delivery (Donn *et al*, 1990). Here success has been restricted to a single, complex genotype. Genotype restriction can be addressed either by trying to broaden the range of genotypes which respond to culture (e.g., Armstrong et al, 1990), or through the exploitation of restriction fragment length polymorphism (RFLPs) and/or other molecular markers, to facilitate rapid introgression of transgenes into target genotypes from culturable material.

There is clearly sufficient precedent described above to believe that transforming Pioneer commercial corn hybrids (registered and marketed in Egypt) with Bt genes to confer insect resistance is an appropriate, feasible and effective biotechnology approach and one that can be pursued with a high expectation of commercial success.

A strong commitment to research, to quality, and to service have been the guides by which Pioneer has built the strong development, production, marketing and sales of corn,

sorghum, sunflower, canola, alfalfa, soybeans, and wheat. Pioneer produces, markets and sells hybrid seed corn, for example, in nearly 100 countries, including through a joint venture, MISR Pioneer Seed Company S.A.E., Nasr City, Cairo, Egypt. (Traditional corn products in Egypt have been of white corn varieties, and the yellow corn market is growing.) Pioneer has a strong interest in the growth and development of improved agricultural biotechnologies which will be essential world wide to meet the needs of the growing world population. Primary plant breeding and research stations are located in 140 locations throughout the world. USAID's ABSP represents an opportunity for Pioneer Hi-Bred to address a target in a developing country in circumstances of shared cost and, as a consequence, lowered commercial risk. There is clear synergy between the goals of USAID and Pioneer Hi-Bred in the development of a market for insect resistant corn in Egypt.

The expected outcome would be for further strengthening of partnering between industry (Pioneer) and Egypt (AGERI) to develop agricultural biotechnology—to develop Bt technologies for maize with resistance to major lepidopteran pests. It is anticipated that the commercial and agronomic success seen with such Bt-maize products in the United States would translate into similar successes in Egyptian agriculture. To succeed will at least require leadership of companies like Pioneer and local education and technology development by institutions such as AGERI.

## Previous Research

The three visiting scientists at Pioneer Hi-Bred have been Mohamed Eid Saad (MES), Mohamed Abd El wahed (MAW), and Gamal Haridy Osman (GHO). Salah Mohamed has been at the University of Wyoming.

MAW made a good start toward maize transformation. Stable transformations with GUS reporter constructs were initiated to test new promoters.

Gamal primarily focused on the study of a novel bacterial isolate to characterize what the insecticidal protein(s) may be. This included the study and implementation of bioassays of larvae on artificial diets, and of standard protein purification and analytical methods.

MES had isolated four or five new putative promoters from maize and linked them to a GUS reporter cDNA for transformation and characterization.

Salah Mohamed (at the University of Wyoming) began by studying the toxicity of a Bt toxin versus lepidopteran pests. His work would include attempting to identify and characterize the Bt-binding protein for this toxin in these insects.

## Specific Project Objectives

### Work Plans for the Project

[ below, (name) indicates location for the work, e.g., (AGERI), refers to work expected to be done at AGERI]**.** For reference, the first twelve months of the project will be considered year 1 of the project, with the subsequent periods considered as years 2, 3 and 4. Please note that years 2 and 3 of the proposed extension are essentially a continuation of the plans made for years 2 and 3 of the original project; the exception being a request for funding of an additional transformation visiting scientist and the addition that Yehia Osman/AGERI will actively screen for and provide additional, new Bt strains.

**End of year 2 targets (cover January 1999 – June 1999)**

***Entomologist Visiting Scientist {Gamal}:***

- Work with the Bt-Receptor Visiting Scientist to refine methods for Bt toxin purification, bioassay the materials, identify the active protein(s), and study binding to insect receptors (Bulla)
- Collect samples of the three targeted maize pests; establish rearing and bioassay conditions (AGERI)
- Begin bioassay screening for new Bts (AGERI)
- Work closely with Egyptian transformation and molecular visiting scientists to build local Bt-maize research and development process (including addressing regulatory agency and intellectual property issues)

***Molecular Biologist Visiting Scientist {Mohamed Eid Saad}:***

- Perform Bt cloning, protein expression and purification, and characterization methods (AGERI)
- Develop molecular methods for Bt-maize in Egypt (AGERI)
- Work with Egyptian transformation visiting scientist to isolate and test new expression vectors (e.g., promoters, selection markers, etc.) (AGERI)
- Develop and implement plan to sample/analyze Bt-maize in Egypt (AGERI)
- Begin the isolation of Bt genes from novel Bts discovered by the Entomologist visiting scientist (AGERI)
- Work closely with Egyptian entomology and transformation visiting scientists to build local Bt-maize research and development process (including addressing regulatory agency and intellectual property issues)

***Transformation Visiting Scientist {Mohamed Abdel wahed} (all at AGERI):***

- Set up maize transformation lab in Egypt
- Attempt to have at least 100 transformation events of Bt genes from this project
- Work closely with Egyptian entomology and molecular visiting scientists to build local Bt-maize research and development process (including addressing regulatory agency and intellectual property issues)
- Work with the molecular visiting scientist to test new maize Bt expression vectors and selectable markers

***Bt-Receptor Visiting Scientist {Salah} (Bulla, Univ. of Wyoming/Univ. of Texas):***

- Identify specific Bt-binding proteins in the target insect pests
- Purify the most likely candidate Bt-binding protein and obtain peptide sequence

***Isolation of Novel Bt Isolates (Yehia Osman at AGERI)***

- Collect and characterize new Bt strains to improve insect control, FTO, and AGERI's intellectual property base
- Bioassay the strains versus target maize pests
- Share the isolates with Pioneer Hi-Bred for purification, identification, cloning the toxins
- Collaborate on the patent and testing process

**End of year 3 targets (cover July 1999 – June 2000) (at AGERI):**

***Entomologist Visiting Scientist {Gamal}:***

- Improve bioassay efficiency, i.e., increased sample test rate, minimal sample used, reliable scores
- Work closely with the other visiting scientists to score transformed Bt-maize

***Molecular Biologist Visiting Scientist{Mohamed Eid Saad}:***

- Develop at least one new proprietary promoter
- Continue testing of new maize Bt-expression vectors, including sampling and analyses
- Isolate and sequence additional Bt genes from this project

***Transformation Visiting Scientists No. 1 {Mohamed Abdel wahed}:***

- Complete at least 200 new events of maize transformed with Bts
- Screen additional Egyptian maize lines for good response to transformation
- Improve transformation efficiency
- Continue to work with the molecular visiting scientist to test new expression vectors
- Work closely with the entomology and molecular visiting scientist to complete sampling and analysis and breeding/crossing of earlier transformed Bt-maize

***Bt-Receptor Visiting Scientist {Salah}:***

- Clone the Bt-binding protein(s) identified in year 2.
- Sequence the clone(s)
- Initiate basic biochemical characterization to verify the recombinant clone encodes a Bt-binding protein
- Submit a manuscript(s) describing the identification and cloning of the Bt-binding protein(s).

## Research Progress

To summarize for 1999 for this report, there has been generally positive progress, and a few changes to the plans.

An extension of the project was arranged to cover the period from September to December 1999. (A request has been made to Pioneer management to consider an additional extension covering the period for the year 2000 and possibly 2001—such extension to at least cover confidentiality for further consulting and correspondence, with the continuance of USAID support unknown and notwithstanding for this period.) Gamal asked for some extension to the end of August 1999. Salah was granted an extension and continued to work with Lee Bulla to the end of August 1999. As of January 2000, Terry has been asked to take on a different role in Pioneer Hi-Bred; with the anticipation (read that as uncertain, but a hope) that he may continue in a consulting capacity for at least the calendar year for this collaboration.

## Progress on end of Year 2 Targets

***Entomologist Visiting Scientist:***

- Gamal spent some time learning and applying methods from Pioneer and from Lee Bulla's lab regarding the identification of insect proteins that bind insecticidal proteins (e.g., Bt-binding proteins). Blots hinted at some binding proteins. Gamal did some PCR cloning of a candidate Bt-binding protein just before he returned to Egypt. Validation of the clone remains to be confirmed (the types of analyses to assess that the clone represents "the" binding protein from the source insect species).
- Hopefully Gamal has been able to work a bit on collecting samples of the three targeted Egyptian maize pests and rearing of them, since his return to Egypt August/September 1999. Likewise, it is anticipated that Gamal will be working with other AGERI scientists regarding building of local Bt-maize research and development process—including isolation and characterization of additional novel Bts.

***Molecular Biologist Visiting Scientist:***

- No work to report in this area from Mohamed for the period. (Mohamed Eid Saad is reportedly fully engaged in graduate studies at AGERI at present.) A patent application was filed by Pioneer regarding some novel plant transcriptional promoters that were isolated by Mohamed Eid Saad with Terry Meyer at Pioneer HiBred. Members of Terry's group at Pioneer have initiated some transient and stable transformation testing of these promoters as GUS-reporter constructs in maize. At least one promoter has shown some interesting GUS staining, but this is from the earliest transformation stages; plants derived from these materials will be further characterized and the results reported.
- Terry forwarded copies of those promoters and of a genomic cosmid library of an AGERI Bt strain to Mohamed. The library was one made by Mohamed while he was at Pioneer. Hopefully, that library will be used by AGERI to isolated candidate Bt genes.

***Transformation Visiting Scientist:***

- I believe Mohamed has been working with Hanaiya's group to make good progress on maize transformation. (Please refer to reports from Hanaiya for any details.)

***Bt-Receptor Visiting Scientist:***

- Salah has been practicing methods from Pioneer and from Lee Bulla's lab for the identification and characterization of Bt-binding proteins. Early work was on the model system Manduca sexta, and more recently with lepidopteran pests. The ligand blots suggested that conditions were developed for detecting Bt-binding proteins on blots of midgut proteins. Salah also made some efforts to try to clone a Bt-binding protein by PCR.

***Isolation of Novel Bt Isolates:***

- Magdy Madkour and Yehia Osman supplied a set of Bt strains from AGERI for study at Pioneer. Members of Pioneer continue to characterize the insecticidal activity of one or a couple of these strains for novel insecticidal proteins. It is anticipated that Yehia will be able to collect additional strains to share in this regard.

- When appropriate, patent applications will be filed on these materials, of course, according to the agreements with AGERI and the USAID project.

## Discussion/Implications

Overall, the project is making progress toward the development of materials and methods for making maize transgenic to control insect pests. The visiting scientists involved in this project have worked hard to learn the key technical aspects of such a project. Experience was provided to them regarding technical, legal (intellectual property rights, IPR), regulatory, and team work. As a collective group, the visiting scientists should be able to coordinate at AGERI the experience and lab tools to design experiments for, to test, to analyze and to characterize maize transgenic for Bt control of insect pests. It is anticipated that Dr. Hanaiya, Dr. Hussein, Dr. Osman and Dr. Madkour will especially guide the next steps of this work at AGERI, with continued discussions with Terry Meyer and Lee Bulla.

The candidate novel promoters that Mohamed Eid Saad and Mohamed Abdel wahed worked on need to be further tested, i.e., in stable transgenic plants to know if they will express transgenes as needed for insect control. Pioneer hopes to wrap up characterization of the available AGERI Bt strains so that any novel materials would become available for in vivo testing. Also, it would be helpful for additional such strains to be provided to Pioneer to aid in scoring them for novel insecticidal activity, and in support of mutual project interests. It may be worth AGERI considering what Bt genes could be used freely in Egypt—i.e., any Bts for which AGERI may have been issued patents, or through continued screening of AGERI materials, or licensed in as appropriate to allow progress in the evaluation of such materials for control of Egyptian maize pests. As I understand it, AGERI has recently hired a gentleman to handle IP work. It's imperative that this person, coupled with the offer of MSU/ABSP to do some patent FTO searching, should seek to identify as early as possible a Bt gene for AGERI to proceed with development of insect-resistant maize. A similar consideration will be good for freedom to operate for maize transformation methods. AGERI has been successful in learning and developing the technical skills to achieve production of Bt-maize for control of Egyptian maize pests.

## Highlights of Significant Achievements

(See comments for the specific visiting scientists, under **Research Progress**.)

AGERI now has in hand various materials and skills to develop and characterize Bt-maize. Gamal, Salah and Yehia are able to purify and clone insecticidal Bts. Mohamed Eid Saad knows how to produce cosmid libraries for cloning of novel genes. Mohamed Abd El wahed is good at transformation of maize, and has support from other members of Hanaiya's group who are also successful in transformation. With some good luck, maybe one of the new transcriptional promoters will prove to have sufficient activity to be useful, and one or more Bt genes may be selected to test in Egyptian maize.

## Publications

Salah completed his Ph.D. dissertation and defense. Mohamed Abd El wahed is nearly finished with writing his dissertation, and will defend it this year. Upon completion of characterizing the maize promoters, there is expectation that publication(s) will be generated.

## Travel

Magdy Madkour, Cathy Ives and Josette Lewis did a site visit to Pioneer in 1999. Gamal and Salah both returned to AGERI around July-August 1999. Terry, Magdy and Cathy met in Washington D.C. in October 1999 to visit about next steps in the collaboration. Terry anticipates visiting AGERI in 2000 to discuss the status and plans for the project. (Terry recently changed jobs within Pioneer, so this visit is uncertain at present.)

## Work Plan for Coming Year

Pioneer Hi-Bred will continue with the transformation and scoring to test the site and relative strength of transcriptional activity from the candidate maize promoters worked on in this project. It is expected that AGERI scientists will likewise work toward testing constructs of these promoters to provide independent assessment of how these materials work under lab conditions at AGERI, and to help the AGERI scientists develop some experience with these materials. Pioneer will reach a point of conclusion in calendar 2000 as to whether the existing AGERI Bt strain(s) indeed has novel activity that merits further cloning and testing or not. It is anticipated that AGERI will continue screening additional Bt strains against key maize pests, and clones of these materials to be characterized. MSU/ABSP has offered to provide some IP (intellectual property) analyses regarding candidate Bt genes for Freedom to Operate (i.e. patent protection issues). Upon identification of a Bt gene with an appropriately potent insecticidal activity and clear IP, AGERI may begin synthesis of a construct for expression and transformation and insect bioassay testing in maize.

# Maize Transformation for Development of Stem Borer Resistance – Egypt

## Research Team

Magdy Madkour
Hanayia El-Itriby
Ebtissam Hussien
Shireen Assem
Mohamed Abdel Sadek
Mohamed Eid Saad

## Overall Project Goals

Maize as one of the major cereal crops in Egypt is cultivated in an area of about 1.8 million acres. Among the insect pests that infect corn plants are stem borers of which Sesamia *cretica* causes most damage as cultural practices, mainly sowing corn fields during mid-May to mid-June, has led to minimizing infestation with *Ostrinia nubilalis* (European cron borer) and restricting its damage to late plantings (July). Application of chemical pesticides has been the only contact measure taken against these insects.

With the commercialization of transgenic Bt crops in the U.S.A., this project was developed to address the following objectives :

1. Transfer technologies from U.S. counterpart to establish a system(s) for regeneration and transformation of Egyptian maize lines.
2. Production of Genetically engineered maize elite resistant to stem borers specifically *Sesamia cretica* (pink corn borer), via transformation with an insect resistance endotoxin Bt gene.
3. Develop laboratory rearing for the lepidopteran pink borer, *Sesamia cretica.*
4. Establish methods for laboratory bioassays and field testing.

## Research Progress

### Establishing a system(s) for regeneration and transformation of Egyptian maize elite lines

#### 1. Maize regeneration

***A. Immature Embryo Culture***

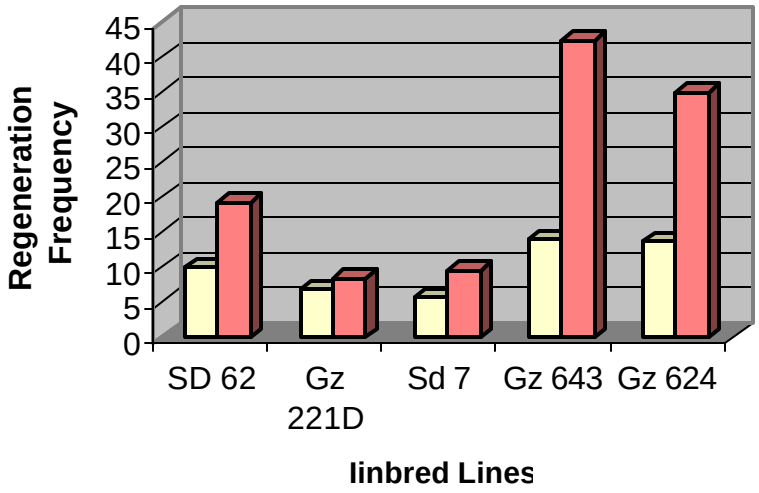
Five inbred lines i.e. (Sd62, G221D, Sd7, Gz643 and Gz624) were used to determine the effect of medium composition and the genotype on the type of callus formed and the regeneration frequency. The average number of embryogenic calli and the regeneration frequency was higher on medium CI-2 that contains N6 medium

supplemented with 100 mg/l myoinositol, 1.0 mg/l 2,4-D, 2.88g L-proline, 1.7mg/l $AgNO_3$ and 2% sucrose as compared to medium CI-1 which was devoid of L-proline and $AgNO_3$. Regeneration data presented in Fig. (1) indicated that the mean number of regenerated plantlets per plate ranged from 1.80 (line Sd 7) to 13.00 (line Gz 643). Accordingly, the lowest regeneration frequency was exhibited by line Sd 7 (5.62%) while the highest regeneration frequency was obtained in line Gz 643 (42.43%).

Lines Gz 643 and Gz 624 gave the highest average number of embryogenic calli (38.1 and 26.3, respectively) and the highest regeneration frequency (42.4 % and 35.0%, respectively).

All genotypes revealed type I callus while callus type II was observed only in Gz 643 on

**Fig. (1): Regeneration frequency of the five Egyptian inbred lines**




*B. Shoot multiplication*

Shoot tips from ten Egyptian maize inbred lines i.e. Sd 63; Sd62; G221D; G336; Rg15; 93/24/91; Sd7; Gz643; Gz624; and Gz630 , were cultured on a shoot multiplication medium SM2 composed of MS medium supplemented with 500 mg/l CH, 0.5mg/l 2,4-D, 2.0mg/l BA and 3% sucrose. The results revealed that significant variation in the shoot multiplication frequency and the average number of shoots formed among the different genotypes. The average number of shoot tips that multiplied varied from 7.7 for G336 to 62.8 for Gz643. Moreover, the lowest numbers of shoots per shoot tip estimated after one, two and three months were obtained by G336 (0.2, 0.3 and 0.3, respectively) while the highest significant numbers were revealed by line Gz643 (3.1, 6.2 and 10.0, respectively), Fig.(1). Therefore, this system proved to be genotype dependent although it was reported as a genotype non-specific system.

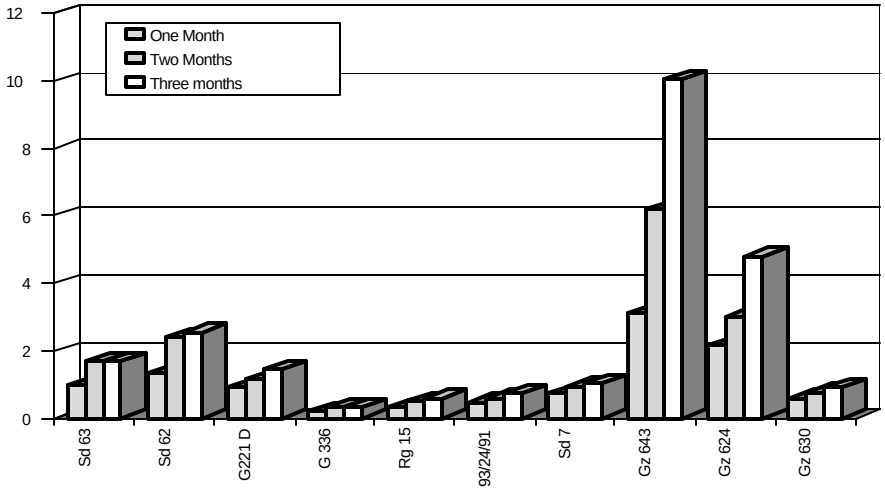



**Fig. (2): Average number of shoot tips per shoot clump after one, two and three months of culture of the ten lines.**

The ability of multiplied shoot tips of line Gz643 to regenerate was tested on two different media. The presence of BA and IBA in the regeneration media enhanced the regeneration frequency of the shoot tips to reach 73.0% as compared to 57.1% on hormone free MS medium, Table (1).

**Table (1) : Regeneration frequency of Gz 643 multiplied shoot tips on different media**

| Inbred Line | No. of shoot tips per shoot clump | Medium M1 and M2 | MS Medium |
|---|---|---|---|
| **Gz 643** | 10-20 | 73.0 % | 57.1 % |

## 2. Maize Transformation

The biolistic particle delivery system was used for transformation of the shoot clumps and immature embryos of line Gz643 by Co-transformation with a combination of plasmids pTW-a (containing the *Bar* gene) and pAct1-F (containing the *GUS* gene) in a (1:1) ratio.

Transformation of immature embryos was carried out using different parameters i.e. osmotic pre- and post-treatment, acceleration pressure (1100 and 1300) and the number of shots applied per plate (one and two). The osmotic treatment of bombarded embryos (4 hours before and 16 hours after bombardment) greatly enhanced the transient expression of the *GUS* gene. The highest average number of *GUS* blue spots was exhibited by embryos receiving 2 shots at 1100 psi. Putatively transformed calli were selected on media containing 1mg/l bialaphos.

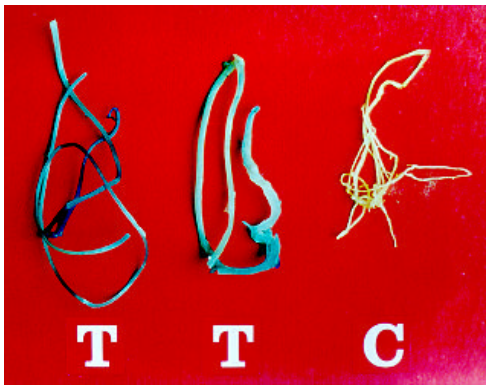
Transformation of shoot clumps was performed using different bombardment parameters i.e. acceleration pressure (1550 and 1800 psi.) and the number of shots applied per bombarded plate (two and four). The *GUS* transient expression revealed that the highest average numbers of blue spots were expressed by shoot clumps bombarded four times at an acceleration pressure of 1550 psi. (363.8). Selection of transformed shoot clumps was performed on media containing 3mg/l PPT.

**3. Analysis of transformants**

Histochemical GUS assay in the transgenic Ro plants revealed the GUS expression in roots of putatively transgenic plants,Fig.(3).

The functional activity of PAT enzyme encoded by the Bar gene was assessed by painting leaves of putatively transgenic plants with 1% BASTA herbicide solution. Herbicide application was performed twice on the putatively transformed Ro plants, Fig.(4).

The herbicide resistant To plants were subjected to different molecular analyses. Eleven and thirteen positive events were recovered from the shoot clumps and immature embryos transformation, respectively, with a transformation frequency of about 1%.




**Fig(3): GUS gene expression in roots of transformed plants (T) and non-transformed control plants (C )**


**Fig (4): Leaves of transformed plants (T) and nontransformed plants (C ) painted with BASTA**

Genomic DNA from leaf samples of putatively transgenic plants resulting from Co-transformation experiments of immature embryos and shoot tip clumps were analyzed by PCR using primers specific to the GUS and Bar coding regions, Fig.(5) &(6).

The integration of Bar and Gus genes was further confirmed by Southern blot hybridization analysis of genomic DNA from leaf samples of putatively transgenic plants, Fig.(7)&(8). The estimated copy no. in the transformed events varied from approximately one to three copies for the Bar gene and 2 to 5 copies for the GUS gene.

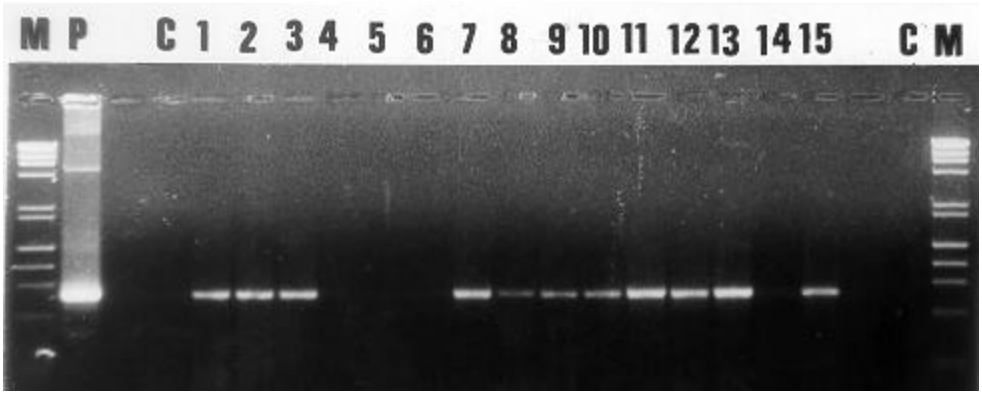



**Fig. (5): PCR amplified DNA for GUS gene in non-transformed and putatively transformed plants**

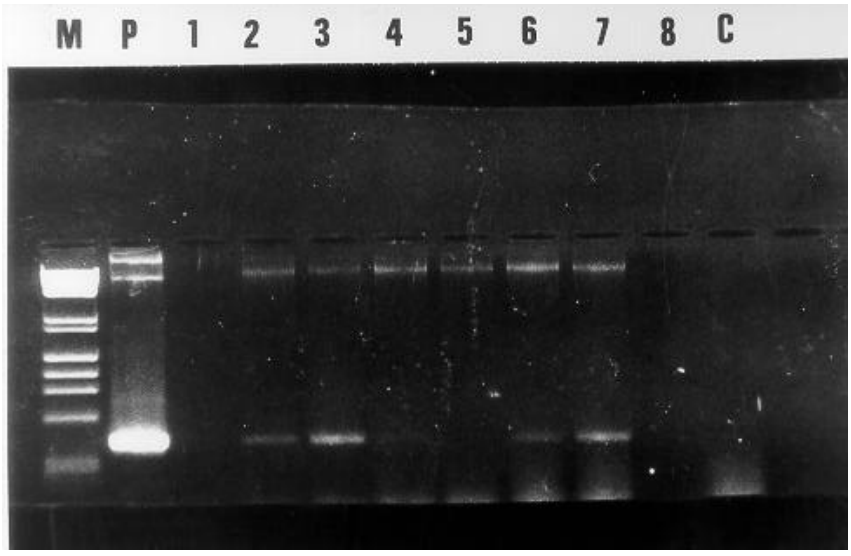



**Fig. (6): PCR amplified DNA for GUS gene in non-transformed and putatively transformed plants**

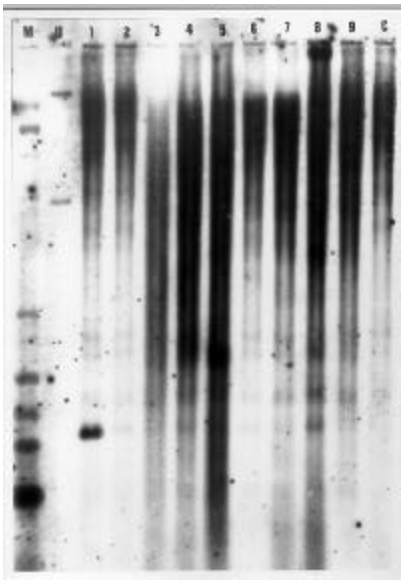


**Fig.(7): DNA hybridization analysis for Bar gene in non-transformed and putatively transformed plants**

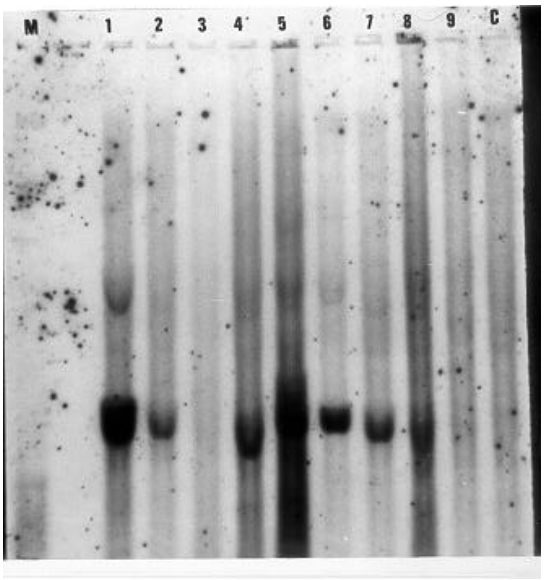



**Fig.(8): DNA hybridization analysis for GUS gene in non-transformed and putatively transformed plants**

**4. Isolation of novel maize promoters**

During Mohamed Eid's training period at Pioneer Hi-Bred, (July97 - July98) he was able to isolate 4 novel maize promoters and a co-patent application file was submitted by Pioneer Hi-Brd to the US-PTO in October 1998.

AGERI received a copy of these promoters in September 99. Mohamed Eid has moved them to new vectors and are currently under investigation to compare their efficiency with other promoters available at AGERI in transformation of Egyptian maize lines.

To isolate new promoters that are FTO, AGERI lacks access to the database that has all the primary information needed to conduct such work. Mohamed was able to get this information through Pioneer databases.

## Publications

A paper was presented at the "Third Arab conference on Modern Biotechnology and areas of Application in the Arab World 14-17 Dec. 1998 Cairo, Egypt. Entitled "Development of efficient regeneration and transformation systems in Egyptian Maize Lines".

## Plan of work (Jan. 2000 - Dec. 2000)

2. Screen additional elite maize lines for their ability in embryogenic callus induction and evaluate their regeneration frequency.

3. Select Embryogenic cell lines from embryogenic cross Sd7 x A188 .

4. Transform lines Gz643 and Sd62 with Bt construct containing gene when available.

# Biosafety Activities

## Principal Investigator

Patricia L. Traynor, Virginia Polytechnic Institute and State University

## Account Number

Subagreement No. 612896; Virginia Tech Account No. 434668

## Overall Project Goals

The ABSP biosafety project is intended to promote the deployment and use of improved crop varieties through short- and long-term technical assistance in biosafety implementation.

## Justification

Biotechnology applications in agriculture continue to progress in developing countries on three continents. In ABSP partner countries, the first research products have been field tested and, in some cases, are moving towards commercial release. Other products, derived from institutional research or being imported from elsewhere, are beginning to enter the biosafety system for approval to conduct field tests or larger-scale releases.

Although many of these countries have scientists highly trained in genetic engineering techniques and conventional agronomic skills, there is a widespread shortage of experts trained in biosafety review procedures. This lack of trained biosafety practitioners will likely delay and may even prevent competent and timely review of applications to test new products. Thus there is a strong potential that insufficient biosafety capacity will impede the transfer of this technology into the hands of farmers and growers. Technical training in risk assessment, risk management, and the biosafety review process is the necessary next step for ABSP support.

## Project Background

During Phase I, ABSP conducted or supported numerous conferences and workshops that dealt with the need for biosafety policy and the preparation and implementation of guidelines. These meetings were instrumental in catalyzing the formulation of national biosafety guidelines in Egypt (1994), Indonesia (1997), and Kenya (1998).

Many of the biotechnology meetings introduced some of the environmental and human health issues raised concerning the release and use of genetically engineered agricultural products. Typically, concepts of risk assessment, risk management, and a step-by-step biosafety review process were presented briefly.

In August 1996, 16 participants from 7 countries traveled to Michigan State for a two-week Biosafety Internship Program. The first week was spent on discussions about

environmental risks and how to identify and manage them. Students were guided through case study evaluations of field test and commercial release applications, acting in the role of National Biosafety Committee members. The second week was spent visiting several seed companies and touring their test plots and field trials of genetically engineered corn, potatoes, poplars, and vegetable crops.

## Rationale

ABSP's support of biosafety awareness and implementation has been and continues to be a fundamental component of the program. It is becoming even more important as GMOs developed in collaborative research projects enter the field testing phase and move towards commercial use. Research progress, coupled with increasing requests from multinational companies to import GMO products, are creating a demand for national biosafety systems that are both operational and credible. In the absence of a functional biosafety system, improved GMO crop varieties will not advance through final testing stages and enter the marketplace.

The logical next step for ABSP assistance, then, is to provide timely support through short-term technical assistance, and to build capacity for making science-based decisions through technical training in risk assessment and risk management.

## Project Objectives with Benchmarks

The ABSP biosafety project has two components. The first is Short-Term Technical Assistance which may include but is not limited to the following tasks:

- participate in national and international conferences and workshops;
- deliver conference presentations;
- consult with policy makers, research administrators, and scientists on matters related to biosafety (defined broadly), including risk assessment, risk management, risk communication, and public acceptance; and
- provide evaluations and reports on biosafety-related topics and materials, as requested by USAID.

The second component is the development of a Biosafety Review Workbook, which is to be used in conjunction with a program of preferably, in-country training workshops. The workbook (and course) will cover:

- the background and context for biosafety review;
- environmental issues associated with the use of GMOs;
- why, when, and where these issues are relevant;
- case studies that allow direct experience with the biosafety review process, using a hands-on approach to realistic cases; and
- sources of supplementary information and support.

The workbook will be accompanied by a teachers' edition containing instructional materials sufficient to conduct the training course independently. It will be distributed in print and on the CABI website.

## Research Progress

Progress in Short Term Technical Assistance:

Presentation on *Biosafety in Agriculture: Ensuring the Environmentally Responsible Use of Biotechnology Products*, Ifrane, Morocco.

- Visit with Moroccan officials to discuss responsibilities and progress in developing biosafety guidelines.

Progress on the Biosafety Review Workbook:

- *completion of concept details and characterization of end product*
- *development of a detailed Table of Contents which shows the scope of material to be covered* (Annex I)
- *recruitment of writers for major sections on Risk Assessment, Risk Management, and Monitoring*
- *identification of several* case study sources for initial release

## Discussion

The Biosafety Review Workbook is designed as an integral part of a technical training course that uses a combination of informal lectures, guided exercises, and case studies to provide hands-on experience in conducting biosafety reviews. Similar technical training is offered through international programs such as UNIDO/ICGEB, however the demand far exceeds the supply of such training courses. Nonetheless, consultations will be made with other providers to minimize duplicated effort.

The Workbook and the companion Instructor's Edition, which includes supplemental teaching materials, is unique to the ABSP initiative. It incorporates a train-the-trainers approach that fosters increased self-sufficiency among course participants. The modular format, with new case studies being added periodically, reflects an ongoing commitment to remaining current with biotechnology products under development.

## Publications

Traynor, P.L. 1999. *Biosafety in Agriculture: Ensuring the Environmentally Responsible Use of Biotechnology Products*. Proceedings of First International Conference on Biodiversity and Renewable Natural Resources Preservation. May 1999, Al Akhawayn University, Ifrane, Morocco.

## Travel

The following project travel was undertaken in 1999:

- Jan 6-10, 1999. Michigan State University. Attend planning meeting with Dr. Josette Lewis (USAID Project Officer), Dr. Andrea Johanson (ABSP Assistant Director), and Dr. Catherine Ives (ABSP Director).

- May 9-16, 1999. Ifrane, Morocco. Participate in biotechnology/biosafety conference and additional discussions regarding Morocco's regulatory system for genetically engineered products.

- June 9-10, 1999. Washington, DC. Give presentations at ABSP Board of Directors Meeting and at BIFAD meeting.

## Workplan for 2000

The following tasks are scheduled for the coming year under the terms of the existing three-year contract for 20% time commitment:

### Short Term Technical Assistance

- attend workshops and conferences, as requested
- prepare talks and presentation materials
- consult with scientists and government officials, as requested
- review biosafety documents from USAID and partner countries, as requested

### Workbook

- identify remaining contributors and their topics
- draft Introduction section
- distribute case study model to writers
- review submitted chapters and case studies
- compile supplemental materials for Instructor's Edition

### Other

- travel to MSU for further planning and review
- prepare quarterly and annual reports, as necessary

# Agricultural Biotechnology for Developing Countries:

# A Workbook for Biosafety Review of Agricultural GMOs

## Draft Concept Paper Prepared for ABSP

P.L. Traynor, May 1999

### Introduction

ABSP biosafety activities under Phase I sought primarily to (1) raise awareness of biotechnology's potential benefits, as well as associated environmental and food safety issues; (2) highlight the need for appropriate government policies; and (3) support efforts to draft national guidelines for testing GMOs in greenhouses and small scale field plots.

Phase II activities are being directed at the next step in biosafety implementation: building capacity for conducting biosafety reviews. This requires technical training that allows guided hands-on practice in applying risk assessment and risk management procedures to crops with genetically engineered traits likely to be introduced into developing countries.

For many countries, biosafety reviews will be needed for field test applications from local public and private sector scientists and representatives of multinational companies, requests from companies seeking to import and sell seed, as well as applications seeking approval for commercial use.

### Intended Audience

The workbook is designed to complement technical training for developing country scientists, Institutional Biosafety Committee (IBC) members, and members of the National Biosafety Committee. It provides supporting information for government biotechnology regulators and monitors. Additionally, it can serve as guidance for IBC members at U.S. academic and public sector institutions as well as reviewers on U.S. government agency (other than USDA) biotechnology committees.

### Objectives

The workbook is being developed in order:

- to provide a structured framework for an ABSP training program for biosafety review committees;
- to build the competence and confidence necessary for biosafety reviewers to conduct science-based reviews leading to appropriate decisions; and
- to develop materials that support ongoing training conducted by local organizations.

**Format**

The workbook is a three-ring binder that will accommodate subsequent updates and additions provided by ABSP; students and instructors will be able to insert their own materials easily. Introductory chapters cover risk assessment and risk management principles, and discuss the leading environmental issues associated with the release of agricultural GMOs. The main part of the Workbook consists of case studies that present realistic examples of field test or commercial release applications, which students are to evaluate under the guidance of experienced instructors.

The cases are modular units; three or four are in the first edition, others are added later as they are developed. A separate instructor's version is supplemented with notes on key concepts illustrated by each case, samples of suitable responses to the guidance questions, pages to be made into transparencies, a bibliography and list of information resources, etc.

**Each case study consists of:**

- a detailed "application" document which describes the recipient crop plant and its biology/ecology, the introduced trait or phenotype, genes and genetic elements and their sources, the field test site, ordinary management practices, relevant information pertaining to potential risks, etc.;
- a series of guidance questions designed to elicit key information;
- a Decision Document on which the student records and justifies the basis for the decision (approved, deferred, or denied), conditions or requirements imposed, and an inventory of the resources required to meet those conditions. To convey the sense of responsibility inherent in decision making, the form includes a signature line.

**In reviewing each case, students document the following steps in their analysis:**

- identify missing information necessary for a scientifically valid review, where applicable;
- determine what risk, if any, is raised; comment on its probability and potential consequences;
- specify suitable risk management procedures that could be applied, if appropriate;
- determine what conditions or restrictions, if any, should be imposed;
- specify monitoring and follow-up procedures, if indicated;
- establish record keeping requirements;
- provide justification for their decision;
- prepare a brief "news release" describing in ordinary language what the GMO is and why it has been found not to pose an unacceptable risk (for approved "applications");
- for denied applications, prepare a brief explanation in ordinary language why it was not approved.

# Information Resources - *AgBiotechNet*

## Principal contacts

Mr David Nicholson, Publisher
Dr David Hemming, Editor
CABI *Publishing*, CAB International, UK

## Summary

The goal of ***AgBiotechNet*** is to deliver current information about biotechnology and biosafety for researchers and policy makers world-wide. The site provides rapid and convenient access to research developments in genetic engineering and updates on economic and social issues. A key element of ***AgBiotechNet*** is the collation of information from diverse sources. The site includes news about corporations, intellectual property rights, technology transfer, biosafety, and bioinformatics. The abstracts section presents a searchable database of the world literature. The review article programme and the books section provide in-depth, critical overviews of the field. ***AgBiotechNet***'s core audience includes scientists and policy makers in developing countries.

***AgBiotechNet*** also features links to related sites, information on patents, detailed spotlight articles on emerging topics, a calendar of events, a jobs section, and an area in which reports and texts from other organizations are published.

## Introduction and Content Development

The full-text content on ***AgBiotechNet*** has been available to users since January 1999. Available content includes:

**News** – updated every two weeks; the news section covers a variety of topics and is international in scope;

**Reviews** – updated monthly with at least two new, specially commissioned articles; section has included a special series on agricultural genomics. The programme is driven by ***AgBiotechNet****'s* Editor (Dr David Hemming) with additional guidance provided by the site's Editorial Board.

Highlights from the Review articles published in 1999 include:

- Factors affecting the adoption of transgenic crops: some evidence from southeastern US cotton farmers (Michele C Marra) ABN 035
- Public acceptance of genetically engineered food in developing countries: the case of transgenic rice in the Philippine (Philipp Aerni) ABN 031

- Agricultural Molecular Biotechnology in South Africa - new developments from an old industry (Ed Rybicki) ABN 023
- The Foundation for African Development through International Biotechnology (FADIB)
- Technology transfer and licensing of agricultural biotechnologies in the international arena (Karim M. Maredia, Frederic H. Erbisch, Catherine L. Ives, Andrew J. Fischer) ABN 017
- The Asian biotechnology market: emerging investment trends (Sachin Chaturvedi) ABN 012
- The DFID plant science research programme: from basic science to the farmers' fields (Roger Hull) ABN 016

**Books** – full-text of eleven titles from CABI *Publishing*. The most recently published titles – *Livestock, Ethics, and Quality of Life*, *Managing Agricultural Biotechnology,* and *Inducible Gene Expression in Plants*, have attracted much interest. The availability of this material in electronic form is unique to *AgBiotechNet* – copies of the printed equivalents are available through standard book distribution channels and direct from CABI *Publishing*. Further books will be published as and when they are published.

The **links**, **patents**, **topics**, and **calendar** sections are all updated on a regular basis and provide comprehensive reference points for existing and new users.

The **links** section now includes a feature which allows users to incorporate a link to ***Agbiotechnet***t on their website. The feature, called link exchange, also allows the user to email cabi with the details of their website so that we can create a reciprocal link to their site.

The **topics** section includes a substantial body of work on a number of important subjects: genetically modified foods, *bt* plants, resistance and other issues, and animal cloning. Each topic section has generated a great deal of interest, demonstrated by the creation of links from other sites on the internet.

The **calendar** section of *Agbiotechnet* is one of the most comprehensive listings of relevant events etc. In this subject area. The service will, in due course, also provide a linkage function to conference proceedings due to be published elsewhere.

**Conferences** – the timely publication of conference proceedings is developing as a key activity for ***Agbiotechnet*.** The first example involved the papers and abstracts from the conference*, From Jay Lush to Genomics: visions for animal breeding and genetics*, May 16-18, 1999, at Iowa State University. The papers were published as .pdf files and the posters as html text.

**Reports** - CABI *Publishing* has developed relationships with a number of organizations generating content in the field. The first such relationship featured the inclusion of ISAAA *Brief* documents on *AgBiotechNet*. To-date *Briefs* 1-10 have been added to the site with more expected soon. The documents are available as .pdf files. These documents have proved to be some of the most popular on *AgBiotechNet*, with several hundred copies downloaded each month. In November 1999 a series of articles commissioned by IFPRI and edited by Gabrielle Persley were added to the site. The next step in this programme will be the addition of the three most recent reports from the National Agricultural Biotechnology Council. The reports focus on the following topics:

- *Agricultural Biotechnology: Novel Products and New Partnerships*

- *Resource Management in Challenged Environments*
- *Agricultural Biotechnology and Environmental Quality: Gene Escape and Pest Resistance*

**Abstracts database** – despite some technical problems experienced in the first half of 1999 the database is now available and as at the end of the year featured over 25,000 records. A major expansion of the database took place in late 1999 with the addition of 8500 records in plant tissue culture. Further expansion of the database is due to take place later this year. CABI may also introduce a new application to host and search the database.

***AgBiotechNet*** has a **listserv** facility which alerts users to new developments e.g. publication of new review articles etc.. The number of registered listserv members has grown to over 200 since the launch of the service.

**Editorial Board** – the growth and development of *AgBiotechNet* is being undertaken with the assistance of a respected and international team of researchers and policy makers. The board currently features 23 members including Dr Catherine Ives of ABSP. New members were added at the end of 1999: Luis Herrera-Estrella of CINEVESTAV, Mexico, and CS Prakash of Tuskegee University.

**AgBio Jobs** – in the last quarter of 1999 CABI developed and implemented a jobs feature on *AgBiotechNet*. The feature allows users to search for positions submitted by a wide variety of academic, government, and commercial organizations from across the world. Submissions are moderated by the Editor. Access to the service and submission of positions is free of charge.

## Access to *AgBiotechNet*

For the year 2000, access to *AgBiotechNet* will be available on the following basis:

1. As a paid-for electronic subscription product available from CABI *Publishing*;
2. As an added value feature of a subscription to the printed journal *AgBiotech News and Information* published by CABI *Publishing*;
3. To our partners in *AgBiotechNet* including the ABSP staff and its grantees;

Transactional, pay-per-view systems for access to *AgBiotechNet* may be introduced in due course.

## AgBiotechNet – Usage Statistics

**Subscriber registrations: 150 institutions and individuals**

| Month | Number of unique user sessions per day |
|---|---|
| January | 87 |
| March | 151 |
| June | 160 |
| September | 136 |
| December | 170 |

**Average user session time:** c.10 minutes January-June; c.45 minutes in July; c.20 minutes in December

User sessions and user session length have built up steadily since the launch of *AgBiotechNet*. The introduction of access controls on the site in October 1999 was a major step in the evolution of *AgBiotechNet*. Much activity in the next 12-18 months will focus on building the community of users able to access *AgBiotechNet* and on ensuring that the controls operate in an efficient manner.

## User Survey

CABI *Publishing* conducted a survey of listserv members in May 1999. Most respondents rated the service as “Excellent” or “Good” and as being of more value than competing services. Further surveys will be carried out on a regular basis to ensure that *AgBiotechNet* continues to meet user needs.

## Conclusion

The contents and user community of *AgBiotechNet* have grown substantially since the products launch in January 1999. The combination of different types of content, from different sources, does, we hope, offer a unique service to our users. This impression is confirmed by user-feedback. CABI *Publishing*’s challenge over the next two years is to build the user community, acquire additional content, and develop linkage capabilities. A key goal for the former is to extend access to *AgBiotechNet* for developing countries on a sustainable basis. Discussions are underway with a number of organizations including the ABSP, ISAAA, IBS, BIO-EARN, and the CGIAR centers. Enhancing the site’s linkage capabilities will involve enabling links from records retrieved during searches of the database through to the corresponding full-text articles (assuming that access controls or pay per view mechanisms are in place), and from full text content in *AgBiotechNet* out to other material on the web.

# Regulation On Biosafety And Food Safety Of Genetically Modified Organisms: Indonesian Experience

## Introduction

Biotechnology, as a new frontier in agricultural sciences, has opened new avenues to the solution of agricultural problems. This technology provides a new and powerful set of tools to address the problems associated with agricultural productivity and environ-mental protection. Genetically improved crop varieties offer the most cost-effective means of increasing yields. Crop yields have been raised significantly through genetic improvements without incurring the risks that go with greater use of chemical fertilizers and pesticides. However, sources of resistance to important pests and diseases are neither easy to obtain nor to introduce into the existing cultivars conventionally. Therefore, advanced technologies must be adopted to achieve the maximum impact from crop improvement programs to increase crop production. Genetic modification of plants using recombinant DNA techniques is promising in increasing crop productivity, product quality, and in reducing dependence on chemical inputs for insect pests control. The promise of genetic engineering in agriculture is related to its ability to manipulate, control and transfer genes in more effective ways. Transgenic plant technology provides dramatic new developments, including an increase in germplasm available for breeding, an increase the genes available for breeding, a decrease in the time necessary for variety development, and expanded plant use for new products and processes (agricultural, chemicals, food processing, specialty chemicals, pharmaceuticals). Despite the clear benefits of genetic engineering in agriculture to produce genetically modified organisms (GMO), there is still some concern on the impact of GMO to the environment, biodiversity, non target organisms and human health. For these reasons a strong biosafety and food safety regulations and guidelines to implement the regulations are needed.

## Current status of research and development of Genetic engineering

The global area of transgenic crops increased by 16.8 million hectares from 11.0 million in 1997 to 27.8 million hectares in 1998. Spain, France, and South Africa, among eight countries, have grown five principal transgenic crops: soybean, corn, cotton, canola, and potato. Transgenic soybean and corn are ranked first and the second. The principal transgenic traits are resistance to herbicides, followed by resistant to insects.

Examples of successful genetic engineering project with plants include resistance to insect pests and diseases. The majority of plants successfully engineered for insect resistances have been transformed with *Bacillus thuringiensis cry* genes, which are either toxic to *Coleoptera* or *Lepidoptera*. Recently, synthetic *cry* genes have been expressed in

high quantities in transgenic plants. Other classes of insect resistant genes such as proteinase inhibitors, trypsins, lectins, and amylase inhibitors were demonstrated to have insecticidal activity.

## Status in Indonesia

Rapid developments in global biotechnology are prompting the Indonesian government to set up a national research program. Increasing yield potential through affecting the fundamental physiological processes governing plant growth may be difficult to achieve through molecular or cell culture techniques. However, these tools may be combined with conventional approaches to provide substantial increases in yield through improvement in individual traits. This belief is supported by the fact that analysis of yield gains due to breeding over the past several decades shows that genetic contribution to yields is primarily the result of added disease, insect, and stress tolerances, all of which can be further improved through the exploitation and further application of biotechnology, especially recombinant DNA technology. The intensity of agricultural production, particularly on food crops such as rice, brings about the build-up of pests and diseases, which become the bottleneck to higher productivity and cause yield instability. These include diseases caused by viruses, bacteria and fungi; insect pests such as brown planthopper and rice stemborer; and rodents. Through conventional breeding, breeders face difficulties in obtaining sources of resistance to insect pests, diseases and environmental stresses.

It is anticipated that techniques in molecular biology will be used to isolate genes, which are proven to be effective against various pests and diseases, and to construct new genes such as viral coat-protein genes. However, research in biotechnology is also a very costly investment and it requires an outstanding group trained and dedicated scientists. Therefore, priority areas of research in this field should be carefully analyzed and identified, and established international collaborations. Most current research activities on genetic engineering focus on developing transgenic plants resistant to insect pest or plant diseases (Table 1). Transgenic plants have been produced through both national and international funding or collaboration (Table 1 and Table 2).

## Biosafety And Food Safety Regulation In Indonesia

Transgenic plants have been successfully developed through international collaboration and various research institutes in Indonesia (Table 1 and Table 2). Some private companies have already applied for permission to test their transgenic plants in Indonesia. Despite the benefit of genetic engineering in agriculture to produce genetically modified organisms (GMOs), there are still some concerns regarding transgenic plants such as whether they have potential to become weeds or be invasive of natural habitats, or potential for gene flow to wild relatives, or whether they have potential impacts on biodiversity, non target organisms, and human health. In order to regulate the safety of GMOs in Indonesia, the Minister of Agriculture signed a decree to regulate GMOS, establishing a biosafety system through the establishment of a national committee and technical team responsible for biosafety reviews and regulations.

## Provisions for Biosafety

The Decree of the Minister of Agriculture No:856/Kpts/HK.330/9/1997 on the Provisions on Biosafety of Genetically Engineered Agricultural Biotechnology (GEABP) was signed by the Minister in 1997. This decree is intended to regulate and supervise the utilization of GEABP to ensure the safety and health of humans, biosafety and the environment in relation to the utilization of GEABP. Whereas, the scope of this decree covers the regulation of the kinds, requirements, procedure, rights and obligations, monitoring and reporting the utilization of GEABP. The kinds of GEABP include transgenic animals, fish, plants, and microorganisms. To implement the Decree No:856/Kpts/HK.330/9/1997, Biosafety Committee and Technical Team for Biosafety were established.

## Biosafety Committee and Technical Team for Biosafety

The Biosafety Committee (BC) was established to assist the Minister of Agriculture. The composition of membership, duties and responsibilities was determined through the Decree of the Minister of Agriculture No: 1038/Kpts/ HK.330/11/97. The BC reviews the application of utilization of GEABP on the technical aspects of biosafety. The Technical Team for Biosafety (TTB) was established to assist the BC through the decree of the Director General of the Agency of Agricultural Research and Development No: HK.330.102.1997. The BC requests the TTB to carry out an appropriate technical study on biosafety of GEABP. The biosafety test of GEABP is conducted in the biosafety containment and confined field trials. The expenses for the biosafety test in the biosafety containment facility and confined fields are charged to the applicant. The TTB is obligated to submit a report on the result of the biosafety tests to the BC. On the basis of the report on the biosafety tests, the BC submits its suggestion/consideration or recommendation to the Minister of Agriculture. The recommendation of BC shall be used as the basis for the determination of whether the application is approved or denied. TTB have developed five series of Guidelines of Biosafety Testing (Plant, Animal, Fish, Microorganism, and General Guidelines).

### Applicant

The applicant, who or which is any person or legal entity must use GEABP and must file a written application by using form model A. Applications are sent to the Minister of Agriculture. The application must be accompanied by the requirements outlined by GEABP. The questionnaires in the application must be filled out and all documents required by the applicant must be supplied. The procedure for applying the utilization of GEABP especially for transgenic plants is described in Figure 1. After the applicant receives the safety statement from the BC, the applicant may apply for multilocation tests for new variety release to the Evaluation and Variety Release Team. The applicant who or which has obtained approval for the utilization of GEABP is entitled to obtain protection for the secrecy of its GEABP with regard to trade or commercial aspects. This is done by keeping certain aspects of the application to the BC and TTB secret.

### Risk Assessment

Risk assessment of GMOs is carried out by the TTB. A risk assessment can be in the form of reviewing and evaluating all documents supplied by an applicant, and conducting biosafety tests in a biosafety containment facility and confined field. The use of transgenic plants must fulfill all requirements, including the production and development

of the plants in Indonesia, or if foreign products, they must pay attention to and take into consideration the religious, ethical, socio-cultural, and esthetical aspects of Indonesia.

### Biosafety Containment

A biosafety Containment facility was built in stages from 1996/1997 till 1998/1999 through the ARM project. The site of the facility is close to the Molecular Biology Division Building of the Research Institute for Food Crops Biotechnology (RIFCB). The first phase of construction, which include a head-house, and three units of greenhouses was completed in 1997. The head-house consists of six rooms including: an *in vitro* culture room, insect rearing laboratory, transformation laboratory, general laboratory, soil preparation room, and staff room. The second phase of construction including two units of greenhouses was finished in 1998. To improve the contained environment in the facility, the connecting-duct between the head-house and the units of greenhouses was constructed in early 1999.

## Biosafety Testing For Transgenic Plants

Biosafety testing for various transgenic plants has been and will be conducted either in the biosafety containment facility and confined fields or in the biosafety containment facility alone (Table 3). The transgenic plants tested to date are: Bt potato resistant to potato tuber moth, Bt corn resistant to corn borer, Bt cotton resistant to bollworm, Bt rice resistant to stem borer, Roundup Ready (RR) corn, cotton, and soybean resistant to glyphosate herbicide, and transgenic peanut resistant to peanut stripe virus. Confined field-testing was conducted in South Sulawesi for BT corn and cotton, and RR corn and cotton, and East Java for RR soybean. A confined field means the field is isolated physically or biologically in distance with the same crops but it is not transgenic.

**The results showed that:**

1. There were no abnormalities with regard to performance or phenotypic characters.
2. There was no tendency to *weediness* that might allow the crop to become a weed of agriculture or to be invasive of natural habitats.
3. There were no adverse human health effects in causing irritation on human skin when compared to non-transgenic plants.
4. There was no impact on non-target or beneficial insects such as parasites, predators, or honeybees.
5. The transgenic plants expressed their traits, i.e. insect resistance or herbicide resistance compared to the non-transgenic crop.

Multilocation tests of some transgenic crops have been and will be conducted for variety release purposed (Table 3).

## Food Safety

Since 1996, an Indonesian Food Law has been implemented. It is stated in Article 13 of the Food Law that food safety testing should be conducted on any food or food products derived from genetically engineered technology before commercialization. In September 1999 the decree of the Minister of Agriculture No:856/Kpts/ HK.330/9/1997 on the Provisions on Biosafety of Genetically Engineered Agricultural Biotechnology was revised to a Joint Decree of Minister of Agriculture, Minister of Forestry and Estate Crops,

Minister of Health, and State Minister of Food and Horticulture No: 998.1/Kpts/OT.210/9/99; 790.a/Kpts-IX/ 1999; 1145A/MENKES/SKB /IX/199; 015A/Nmeneg PHOR /09/1999 as "the **Biosafety and Food Safety of Genetically Engineered Agricultural Products"**. Biosafety Committee was reformed as the Biosafety and Food Safety Committee, and the Technical Team for Biosafety will be reformed as Technical Team for Biosafety and Food Safety. Guidelines for Food Safety Testing on GMOs have been drafted.

## Statement of Safety

There will be three types of safety statements:

1. **Biosafety** and **Food Safety**: safety statement for a kind of crop grown in Indonesia and used for foods and feeds, such as corn, soybean, rice, etc.
2. **Biosafety**: safety statement for a kind of crop is grown in Indonesia and not used for foods and feeds, such as cotton.
3. **Food Safety**: safety statement for imported grain or seeds are used for foods and feeds but not grown in Indonesia, such as imported corn or rice grain or soybean seeds.

## Public Awareness

Public awareness of GMO and the regulation of Biosafety and Food Safety of GMO have been carried out by various research institutes and universities throughout Java island and South Sulawesi. Two days of training on Biosafety and Food Safety of GMOs for research scientists have also been conducted at the Research Institute for Food Crops Biotechnology.

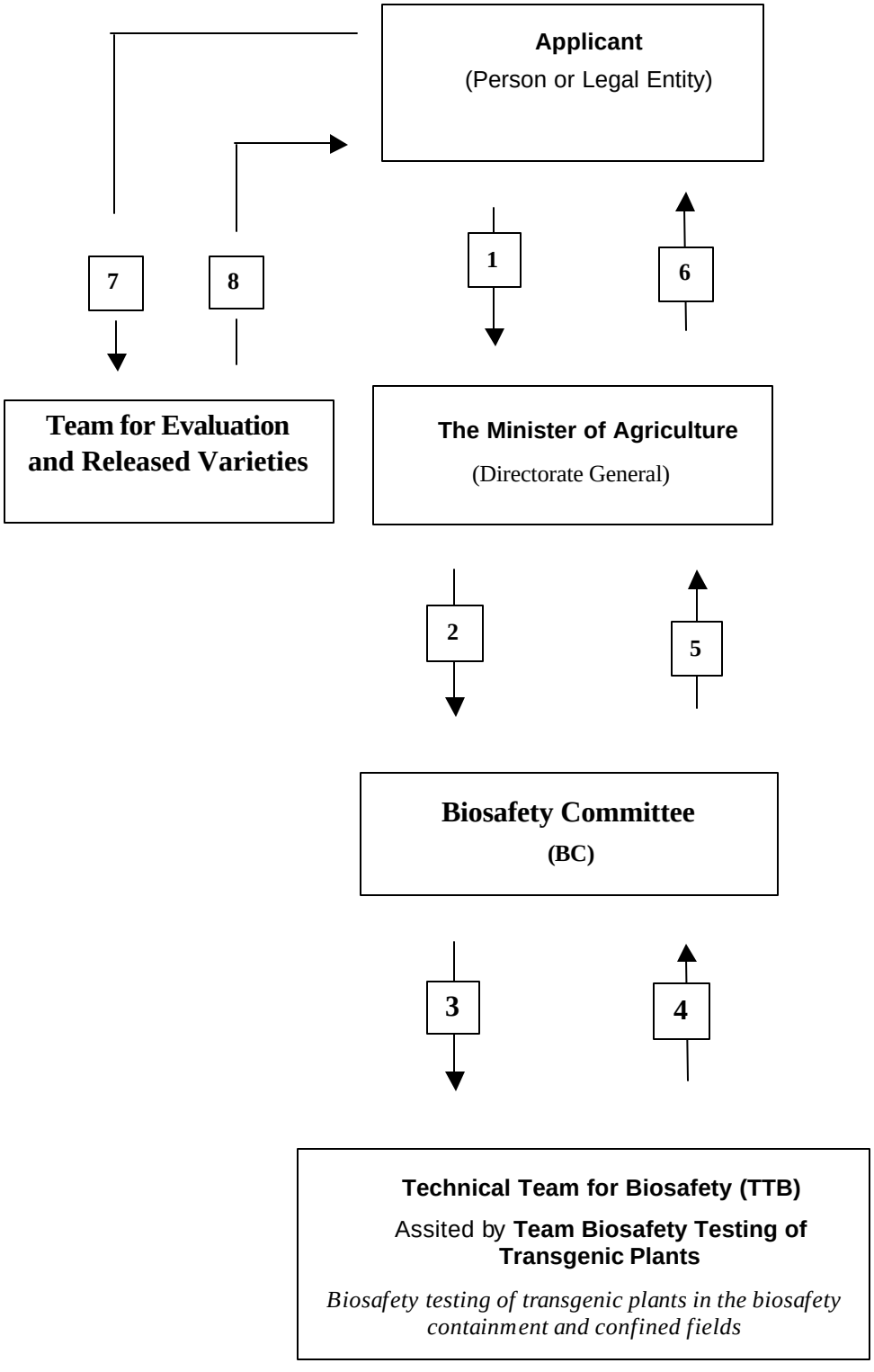



**Figure 1. Procedure for utilization of transgenic plants**

Note:

1. = Application for utilization of transgenic plants
2. = Request for technical suggestion on utilization of transgenic plants
3. = Request for biosafety testing of transgenic plants in the biosafety containment and confined fields
4. = Suggesstion on the utilization of transgenic plants
5. = Recommendation on the utilization of transgenic plants
6. = Denial or approval of the application for utilization of transgenic plants
7. = Application for varieties release of transgenic plants
8. = Denial or approval of the varieties release of transgenic plants

**Table 1. Research Activities On Development Of Transgenic Plants In Indonesia**

| No | *Crops* | Traits | Gene | Institution |
|---|---|---|---|---|
| 1 | ***Corn*** | ***Resistant to ACB*** | ***Pin II*** | ***RIFCB*** |
| 2 | Peanut | Resistant to Pstv | CP | RIFCB and BAU |
| 3 | Cacao | Resistant to borer | Bt | BRUEC |
| 4 | Soybean | Resistant to pod borer | Pin II | RIFCB |
| 5 | ***Rice*** | ***Resistant to RSB***<br>***Resistant to RSB and BPH*** | ***Bt***<br>***Bt & GNA*** | ***RIFCB***<br>***CRIB, IIS*** |
| 6 | Sugarcane | Resistant to SSB | Bt | ICRIS |
| 7 | ***Tobacco*** | ***Resistant to TMV*** | ***CP*** | ***RITFC*** |
| 8 | Sweet potato | Resistant to weevil | | RIFCB |
| 9 | Sweet potato | Resistant to SPFMV | | RIFCB |

Italic and bold letter indicated that transgenic crop has been produced (others indicate ongoing research). ACB = Asian Corn Borer; BAU = Bogor Agriculture University; BPH = Brrown Plant Hopper; BRUEC = Biotechnology Research Unit for Estate Crops; Bt = *Bacillus thuringiensis*; CP = coat protein; CRIB = Central Research Institute for Biotechnology GNA = *Galanthus nivalis* agglutinin (snowdrop lectin); ICRIS Indonesian Central Research Institute for Sugar; IIS = the Indonesian Institute for Sciences; Pin II = proteinase inhibitor II; Pstv = peanut stripe virus; RIFCB = Research Institute for Food Crops Biotechnology; RITC = Research Institute for Tobacco and Fiber Crops; RSB = Rice Stem Borer; SPFMV = Sweet Potato Feathery Mottle Virus; SSB = Sugarcane Stem Borer; TMV = tobacco mosaic virus;

**Table 2. Transgenic crops developed through international collaboration**

| No | *Crops* | Traits | Gene | Institute |
|---|---|---|---|---|
| 1 | Corn | Resistant to ACB | Bt | RIFCB/CRIFC/ICI Seed Co. |
| 2 | Peanut | Resistant to Pstv | CP | RIFCB/CRIFC/ACIAR |
| 3 | Potato | Resistant to PTM | Bt | RIVC/RIFCB/CRIFC/ MSU |
| 4 | Sweet potato | Resistant to SPFMV | CP | RIFCB/CRIFC/Monsanto |

ACIAR = Australian Center for International Agricultural Research; Bt = Bacillus thuringiensis; CP = coat protein; CRIFC = Central Research Institute for Food Crops; MSU = Michigan State University; Pstv = peanut stripe virus; PTM = potato tuber moth; RIFCB = Research Institute for Food Crops Biotechnology; RIVC = Research Institute for Vegetable Crops; SPFMV = sweet potato feathery mottle virus.

**Table 3. Status of biosafety test for transgenic crops in Indonesia**

| No | Crops | Traits | Institution/ Private Co. | Biosafety containment test | Confined field test | Multilocation tests |
|---|---|---|---|---|---|---|
| 1 | Bt corn | Resistant to ACB | Pioneer | Being conducted | – | – |
| 2 | Bt corn | Resistant to ACB | Monsanto | Were conducted | Were conducted | – |
| 3 | Pin II Corn | Resistant to ACB | RIFCB/ABSP | Being conducted | – | – |
| 4 | RR Corn | Resistant to *glyphosat* | Monsanto | Were conducted | Were conducted | Were conducted |
| 5 | Bt cotton | Resistant to CBW | Monsanto | Were conducted | Were conducted | – |
| 6 | RR cotton | Resistant to *glyphosate* | Monsanto | Were conducted | Were conducted | – |
| 7 | Peanut | Resistant to Pstv | RIFCB/ACIAR | Will be conducted | – | – |
| 8 | RR soybean | Resistant to *glyphosate* | Monsanto | Were conducted | Were conducted | Will be conducted |
| 9 | Bt potato | Resistant to PTM | RIVC/RIFCB/ CRIFC/MSU | Were conducted | Will be conducted | – |
| 10 | Bt and GNA rice | Resistant to RSB and BPH | CRIB, IIS | Being conducted | – | – |
| 11 | Bt rice | Resistant to RSB | RIFCB | Being conducted | – | _10 |

ABSP = Agricultural Biotechnology for Sustainable Productivity; ACB = Asian corn borer; ACIAR = Australian Center for International Agricultural Research; BPH = Brown Plant Hopper; Bt = *Bacillus thuringiensis*; CBW = Cotton Bollworm; CRIB = Central Research Institute for Biotechnology; CRIFC = Central Research Institute for Food Crops; GNA = *Galanthus nivalis* agglutinin (snowdrop lectin); IIS = the Indonesian Institute for Sciences; MSU = Michigan State University; Pstv = peanut stripe virus; PTM = potato tuber moth; RIFCB = Research Institute for Food Crops Biotechnology; RIVC = Research Institute for Vegetable Crops; RR = Roundup Ready; RSB = Rice Stem borer.

# REPORT ON THE WORKSHOP:

# "THE IMPACT OF INTELLECTUAL PROPERTY RIGHTS ON INTERNATIONAL TRADE AND AGRICULTURE IN EAST AFRICA"

**JANUARY 18-20, 1999, KAMPALA, UGANDA**

## Background

The workshop was held at the Sheraton Hotel, Kampala, Uganda from January 18-20, 1999. The workshop was organized by the Agricultural Biotechnology Support project (ABSP) based at Michigan State University and the Ugandan Council for Science and Technology (UNCST). The United States Agency for International Development (USAID) supported the workshop, and additional funds for participants were obtained from the Technical Center for Agricultural and Rural Cooperation (CTA, Netherlands), the Rockefeller Foundation and Monsanto.

### OBJECTIVES

As a consequence of The Agreement on Trade Related Aspects of Intellectual Property Rights (TRIPS) of the WTO, and the Biosafety Protocol, developing countries will be pressed to establish a biotechnology policy framework that includes both intellectual property (IP) rights protection and biosafety regulations. One of these IP requirements is the establishment of protection of intellectual property rights for plant varieties. Most of the African countries have not yet met this requirement. Both policies also play a critical role in promoting the transfer and application of biotechnology to developing countries.

The aim of the workshop was to assist the participating African nations in building background and expertise in the intellectual property aspects of biotechnology, particularly in agriculture. It aimed to provide an effective forum for the discussion of the importance of intellectual property policies for the development of agricultural biotechnology in Africa, and the impact of such policies on the availability of, and trade in, agricultural biotechnology products.

The workshop was intended for officials from government intellectual property organizations, and from the scientific organizations developing biotechnology-derived products, as well as for those in the private sector agricultural and legal communities. It covered the variety of intellectual property systems of importance to biotechnology, the administrative implementation of the systems, relevant international issues including those associated with export agriculture and genetic resources, and the commercialization of publicly developed technologies. Presentations were given by African, US and European experts in intellectual property rights and international trade (speaker list attached).

The overall goals of the workshop were: i) to create better understanding of the policy issues surrounding agricultural biotechnology; ii) to develop linkages between the national scientific and legal communities, and; iii) to develop improved intra-regional linkages.

## Workshop participants

Over 70 participants attended the workshop (participant list attached). Countries represented were: Ethiopia, Kenya, Nigeria, Tanzania, Uganda, Zambia, Zimbabwe, Switzerland, United Kingdom, France, United States, Costa Rica, South Africa, and the Netherlands. Participants came from a broad range of backgrounds - from the legal profession and the national offices

responsible for patents and plant variety protection, from the private sector agricultural companies, and from national agricultural research institutes. Many of the participants were high level representatives of their institutions, but there were also scientists from the national research programs with a direct (and often urgent) practical interest in how to take forward the results of their research work.

## Program

The workshop program covered a number of issues ranging from Intellectual Property Rights (IPR) and the Trade Related Property Rights (TRIPS) agreement to the International Convention for the Protection of New Plant Varieties (UPOV). Regional country representatives also gave presentations on the current situation in their country with regard to agricultural biotechnology, and the current national status of IPR and PVP laws. On the final day of the workshop the program was adjusted to allow for participants to break into four working groups in order to address issues that had arisen in the previous two days. Summaries of the main points raised and recommendations made are given in the Appendix. The full program for the workshop is attached.

## Outcomes and Comments

Bringing together such a diverse group of participants was one of the aims of the workshop, however, this also meant that people's level of knowledge of the different issues varied greatly, and that their goals in attending were very different. Feedback given to the organizers by the participants was very positive, with the majority of people agreeing that this was a vitally important issue and that more discussion on the topic was urgently needed in Africa.

Discussion sessions were very lively, indicating the high level of interest and not insignificant level of controversy surrounding some of the concepts of IPR and biotechnology. In the initial discussion sessions some participants communicated strong feelings of suspicion and even fear of biotechnology (specifically in agriculture, not necessarily in the medical/pharmaceutical area), of the multi-national companies whom they see as forcing the technologies on them, and the international trade organizations and the treaties they produce. Much of this reaction seemed to stem from debate surrounding Monsanto's so-called 'Terminator Technology' (Gene Protector). Interest in this subject was high and there is a genuine concern in developing countries that this technology may pose a serious threat both to farmer's rights to save seed, and to non-transgenic crops in the area which may be cross-pollinated causing germination failure in the next generation of seed. In response to these expressed concerns, Monsanto produced a handout, and gave a short presentation on the current status and future possibilities of this technology. According to Monsanto the Gene Protector technology is not likely to become commercially available until 2005 at the earliest.

From much of the discussion it appeared that although people may be aware of some of the possible problems associated with biotechnology, the scientific community has not done enough in stressing the potential advantages. Many of the discussions were based on the premise that there were no real advantages to biotechnology, but that it would be forced upon developing nations by the multi-national companies, and that choice would therefore be removed from farmers. Most countries do possess national registration systems that give each country the option of approving or not any agricultural product. Several of the representatives from the NARS emphasized some of the advantages that biotechnology could bring to African agricultural systems, for example crops with pest and disease-resistance, drought and salt-tolerance, and improved protein and nutrient content. For example, improved varieties of maize are already available and might be useful in Africa. Currently due to the lack of IP policies in Africa there is little incentive for companies to consider research in improving other African crops.

Many of the developing country representatives raised ethical/moral objections to the principle of patenting life forms. This view is widespread and is holding back the development of IP legislation in many regions. Although the cultural and religious reasons for this are complex and should be acknowledged, the fact remains that investment in biotechnology is driven by the concept of intellectual property. Some resolution of this conflict is essential to ensure that technology is transferred from the developed to the developing world. Another concern expressed was the inaccessibility of patents. This is not such a problem in the USA where it is possible to access an online database containing details of all patent submissions. It was stressed, however, that the US model should not necessarily be copied in developing countries. It is possible to devise a system that is similar, but more appropriate to the needs of developing countries -- one that is more accessible, less costly and that would put less of an administrative burden on a country with limited legal and scientific resources. Regional harmonization of patent responsibilities may also be a practical option.

Related to this, the issue of the ownership of indigenous technologies and germplasm was occasionally raised in the discussions. Although this workshop was not specifically geared to these issues and others raised by the Convention on Biological Diversity (CBD), this was discussed briefly. It is still unclear how countries will benefit from these agreements, and there is an apparent contradiction in the views of some of the developing countries with respect to this -- wanting to hold rights over indigenous technology and germplasm, yet not accepting other implications of IP.

Most of the countries represented do not currently have very high capacity in biotechnology., although Kenya and Ethiopia are perhaps the most advanced. However, many of the NARS do have some capacity for tissue culture and basic molecular biology capabilities, and are keen to develop in this area. Many NARS have collaborative projects with European and US public sector institutions, but are restricted from progressing far with research due to the lack of IP and biosafety legislation. Perhaps due to this low level of capacity in biotechnology, the area of most interest was that of plant variety protection.

## FOLLOW-UP & RECOMMENDATIONS

- One of the most important factors in developing policies that will encourage the development of biotechnology policies is basic scientific capacity building. One of the reasons that there is concern expressed by developing countries is that they do not have the scientific capacity to carry out the research themselves, or have the resources to invest in any regulatory activities that may be required. It is important that developing countries are able to obtain their own IP in order to trade from a position of strength with the private sector in the US and Europe.

- Initially, it seems logical to focus on plant variety protection (PVP), as this seems to be the way most of the countries want to go. Capacity building efforts will be required for countries to be able to implement PVP, and assistance will be required from UPOV to draft the appropriate legislation. The 2005 deadline for WTO compliance with TRIPS (i.e. the extension of IP protection to plant varieties) is forcing developing countries to make this a priority.

- It is important to note however that PVP alone may not encourage the biotechnology industry to invest in developing countries. It may act to encourage local plant breeding efforts, a very different outcome, but perhaps a good first step on the road to developing IP policy. However, there is the possibility that without sound patent reforms, Africa may miss out on cutting edge technology such as transgenic crops, human and animal vaccines, and possibly even new pharmaceuticals. Efforts must therefore be made to encourage the processes of cooperation and policy reform being undertaken by African countries to improve agricultural trade and technology access.

- A regional approach to IP issues is likely to be the most effective. Uganda, Tanzania and Kenya seem committed to UPOV legislation, and Kenya, being the most advanced in this area, could provide important lessons to the other two countries. In addition, regional harmonization will result in better use of human capacity in biotechnology and IP issues in the region.

- IP systems (PVP/patents) must be designed both nationally and regionally, as there is little market incentive for companies to move into one country unless there was a regional market. Systems must be established that will assist African countries in obtaining access to technology, not cut them off from its benefits.

- It is vital to continue to cooperate in capacity building and in sharing tools and knowledge with African scientists and policy-makers. Continued interactions must be maintained in order to explore and discuss the importance of these issues in international trade agreements.

- Efforts in the development of IP policies must be accompanied by sound policy, law and business training.

- Market access must be ensured in the export nations - this will need not only appropriate IP protection legislation, but also more attention must be given to international grades and standards.

## CONCLUSIONS

In order to take advantage of the international trade agreements, policy reforms such as the application of IPR to agriculture must be addressed in Africa. This is particularly important because the agricultural sector continues to play a key role in both food security and economic growth in the region. Improvements in agricultural production through the application of biotechnology will contribute to both these aims. Africa will not always be an importer of this technology -- greater economic benefit from biotechnology will be realized when African countries co-develop and apply biotechnology based on their local agricultural systems and genetic resources. There is some commitment to starting the process of policy reform, but this must be encouraged by continued education and capacity development in the region.

**APPENDIX**

# Reports from Working Groups

## I TECHNOLOGY TRANSFER ISSUES

Different types of science and technology transfer mechanisms were acknowledged:

- institutional capacity building
- human
- physical
- funds

Coordinated National and regional strategies are needed.

**Problems with technology transfer:**

- inadequate funding
- inadequate government commitment
- inadequate influence on policy implementation
- brain drain

**POSSIBLE SOLUTIONS:**

- technologies must be appropriate
- develop local technologies where possible
- impress on government the importance of technology & obtain increase funding
- develop more collaborative partnerships with both private and public sectors (important at all levels)
- be proactive with ideas for private sector – a two-way process
- technology sales/promotions desirable

## II UPOV requirements and implementation

- The entire group was happy with the 1978 ACT.
- The 1991 Act was considered to be interesting for African countries, particularly with regard to the essential derivation principal.
- It was agreed that a major task of explanation and education was required in order to familiarize agricultural circles with the issues.
- It would be useful to identify a focal point for legislation, preferably in collaboration with the seed industry.
- Participants agreed that the UPOV office should be asked to co-operate with individual country legal professionals in the preparation of legislation.
- It is possible for a country to have a national protection act even before joining UPOV
- It was agreed that plant varieties should be excluded from patent protection.
- It was suggested that countries introducing protection for the first time should take advantage of the opportunity to provide protection of existing varieties by waiving the novelty requirement.

- The group felt that there were no regional mechanisms for discussion of issues relating to international trade and agriculture in East Africa. It was recommended that countries in the region should develop a forum for future discussions of PVP -- particularly capacity building.

## III International Agreements

- Participants expressed the concern that African views and interests are not generally well represented in the international arena e.g. WTO, UPOV, GPA, IU, CBD.
- Capacity and interest do exist in Africa, but these are often scattered and incoherent, even at the national level. Coordination in approach is needed.
- There is a need for the generation of cross-sectoral debate, both nationally and regionally to create awareness and to encourage interest and involvement of national representatives/negotiators.
- There should be an active push for a comprehensive review of international agreements that will allow for the creation of more equitable agreements.
- Possible first step – creation of an email 'listserver' in Africa for information, contacts, discussion etc.

## IV Patent Systems for Africa

Is there a model system that African countries can adopt?

- The inventive step for a patent may be wide or small. It was agreed that if this step was made larger, a lower number of higher quality patents would be approved and the process therefore made easier.
- It is possible to set up a system of 'petit' patents for local inventors. These have slightly different criteria for approval -- smaller inventive step, less costs to file etc.
- A research exception can be included for academic research purposes (however, if research subsequently leads to commercialization the licensor must then be paid).
- Different fee structures can be explored: for example, in South Africa there is no formal patent examination system, patents are valid unless challenged in court. It is possible to set up a system where there are different levels of fees – e.g. where larger companies pay more than smaller companies and non-profit organizations.
- However, when patenting abroad there is no option - it is not possible to discriminate between filers.

# WORKSHOP PROGRAM

# The Impact of Intellectual Property Rights on International Trade and Agriculture in East Africa

**JANUARY 18-20, 1999 KAMPALA, UGANDA**

## Monday January 18

**INTRODUCTION**

9:00 – 11:30 Welcome and statements by host-nation and East African officials.
Welcome and statements by US officials.

*11:30 – 12:00* *Break*

**INTELLECTUAL PROPERTY ISSUES BEFORE EAST AFRICA IN THE BIOSCIENCES**

12:00 – 12:50 Introduction to intellectual property in the bioscience context and introduction to the international context of intellectual property laws.
**John Barton, Stanford Law School**

*12:50 – 14:00* *Lunch (& roundtable discussion)*

14:00 – 15:00 National presentations on breeding and research activities and international trade in agricultural and biological products
**Regional Participants**

15:00 – 15:30 Discussion

*15:30 – 16:00* *Break*

**LEGISLATION AND ADMINISTRATION FOR PLANT VARIETY PROTECTION**

16:00 – 16:45 Introduction to Plant Variety Protection & establishment of a PVP office.
**Barry Greengrass, UPOV**

16:45 – 17:30 Discussion

## Tuesday January 19

**LEGISLATION FOR PATENTS IN BIOTECHNOLOGY**

9:00 – 9:40 Use of regular patents to protect biotechnological inventions.
**John Barton**

9:40 – 10:30 Operational requirements for a patent office.
**Michael Blakeney, University of London**

10:30 – 11:00 The role and operation of ARIPO.
**Mzondi Chirambo, ARIPO**

*11:00 – 11:30* *Break*

11:30 – 12:30 National reports on PVP and patent issues in agriculture in East Africa.
**Regional Participants**

12:30 – 12:50 Discussion

*12:50 – 14:00* *Lunch (& roundtable discussion)*

**TECHNOLOGY TRANSFER ISSUES**

14:00 – 15:00 The role and process of technology transfer -- a university perspective.
**Rosemary Wolson, University of Capetown/Silvia Salazar, Costa Rica**

15:00 – 15:30 Discussion

*15:30 – 16:00* *Break*

16:00 – 17:00 The role and process of technology transfer -- a business perspective.
**Judy Chambers, Monsanto**

17:00 – 17:30 Discussion

**Wednesday January 20**

**INTERNATIONAL TRADE ISSUES**

9:00 – 9:30 Introduction to TRIPS and international conventions.
**John Barton**

9:30 – 10:10 TRIPS and the Convention on Biological Diversity.
**Robert Lettington, ACTS**

10:10 – 11:00 Intellectual property developments and market access in important market nations.
**Steve Collins, Monsanto/Jean Donnenwirth, Pioneer Hi-Bred International**

11:00 – 12:50 Discussion on international Intellectual Property issues

*12:50 – 14:00* *Lunch (& roundtable discussion)*

14:30 – 15:30 Break out sessions:

i. Technology transfer/technology access (**Salazar, Wolson, Chambers**)
ii. UPOV requirements and implementation (**Greengrass**)
iii. International agreements/conventions (**Lettington**)
iv. Patent systems for Africa (**Barton, Donnenwirth**)

*15:30 – 16:00* *Break*

**CONCLUDING SESSION**

16:00 – 17:00 Plenary – summary of discussions, future needs.
17:00 – 17:30 Closing Ceremony